### 1. Alzheimer's Disease Research
- Human β-Amyloid (1-40) ELISA Kits
- Human/Rat β-Amyloid (40) ELISA Kits
- Human β-Amyloid (1-42) ELISA Kit & the High Sensitive Kit
- Human/Rat β-Amyloid (42) ELISA Kit & the High Sensitive Kit
- Amyloid β-Protein Immunohistostain Kit

### 2. Antibodies
- Anti Ago2, MAb
- Anti Human CETP, MAb
- Human CETP ELISA Kit
- Anti Iba-1
- Anti Olfactory Marker Protein [Anti OMP]
- Anti Phosphorylated
- Anti soluble Guanylate Cyclase (sGC); the NO insensitive, MAb
- Anti SQSTM1 / A170 / p62
- Anti Agt-1; Anti Rat VGLUT-1; Anti Rat VGLUT-2
- Anti Substance P
- Anti Iba-1
- Anti Lysotracker Red DND-99, MAb

### 3. Apoptosis Research
- Apoptosis in situ Detection Kit Wako
- Apoptosis Screening Kit Wako
- Camptothecin
- Cytotriotenin A
- ETB [Epolaectane Tertiary Butyl Ester]
- RKTS-33

### 4. Cell Separation
- Iron Powder, from Iron Carbonyl
- Nylon Fiber & the columns

### 5. Chemiluminescence Probes
- Green Chemiluminescent CD
- L-012

### 6. Fluorescent Probes
- BES-H₂O₂-NT-Ac
- BES-H₂O₂ (Cell impermean) -based on a non-oxidative mechanism
- BES-So-AM
- BES-So-Ceimpermeant
- BES-Thio
- DAMPAQ-22
- KMG-20-AM

### 7. microRNA Research
- microRNA Cloning Kit
- microRNA Isolation Kit, Ago2
- Single Strand DNA Ligase, thermostable, recombinant, Soln.
- Anti Ago2, MAb

### 8. Physiological Active Substances
- 17-AAG < Apoptosis Inducer / HSP90 Inhibitor
- ADP-Ribosyltransferase C3 < Botulinum neurotoxin C3 w/o toxicity
- Ampicillin & the Na Salt < Synthetic penicillin
- Anisomycin < Apoptosis Inducer
- Aristeromycin < AMP synthesis inhibition
- Bafilomycin A1 < Vacular H⁺ ATPase Inhibitor
- Bleomycin Hydrochloride < Anticancer antibiotic
- Bleomycin Sulfate < Anti Cancer antibiotic
- α-Bungarotoxin < a neurotoxin binds to acetylcholine receptors
- Calyculin A < Neurotoxin; Protein Phosphatase inhibitor
- Capsaicin < Neurotoxin; Prototype vanilloid receptor agonist
- Carbenicillin Sodium Salt < bacterial cell-wall synthesis inhibitor
- Chloramphenicol < blocking the peptidyltransferase reaction
- Ciclosporin A < Potent immunosuppressive agent
- Concanamycin A < Vacular H⁺ ATPase Inhibitor
- Deguelin < Akt (Protein Kinase B) inhibitor
- Dinophysistoxin-I [DTX-I] < Protein Phosphatase inhibitor
- ETB < New HSP60 Inhibitor / Apoptosis Inducer
- Gentamicin Sulfate < bacterial protein synthesis inhibitor
- GGsTop™ < New -Glutamyl transpeptidase (GGT) inhibitor
- Idebenone < a synthetic analog of Coenzyme Q10
- Joro Spider Toxin [JSTX-3] < Neurotoxin; Glutamate Receptor Inhibitor
- Kainic Acid n-Hydrate < Neurotoxin; Glutamate Receptor Agonist
- Kanamycin Sulfate < Protein synthesis inhibitor
- (+)-MK801 Maleate < NMDA-Glutamate Receptor Antagonist
- Mycalolide B < Actin Inhibitor
- Nobiletin < Polymethoxy flavonoid derived from Shekwasha
- Okadaic Acid & the Ammonium Salt < Protein phosphatase inhibitor
- Palytoxin < Neurotoxin; Na⁺ Channel Agonist
- PAQ-22 < Puromycin-sensitive aminopeptidase (PSA) inhibitor
- Reveromycin A Sodium Salt < Protein Synthesis Inhibitor
- RKTS-33
- Ryanodine < Potent inhibitor of Ca²⁺ release
- Stellettamidine A Trifluoroacetate < Calmodulin inhibitor
- Tangeretin < Polymethoxy flavonoid derived from Shekwasha
- Tautomycin < Protein phosphatase inhibitor
- Trichostatin A < HDAC Inhibitor
- Tryprostatin A < Antibiotic
- Xestospongicin C < IP3 receptor inhibitor
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<td>Xestospongin C</td>
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for quantitative determination of \( \beta \)Amyloid peptide 40 and 42

**\( \beta \) Amyloid ELISA Kits**

Alzheimer’s Disease (AD) is characterized by the presence of extracellular senile plaques (SPs) and intracellular neurofibrillary tangles (NFT) in the brain. The major protein component of SPs is \( \beta \) Amyloid peptide (A\( \beta \)) 40 and 42(43). A\( \beta \)42 is more prone to aggregate than A\( \beta \). Therefore the initial A\( \beta \)42 deposition begins with A\( \beta \)42(43) but not with A\( \beta \)40. A\( \beta \)42(43)-positive and A\( \beta \)40-negative plaques may represent early-stage diffuse type SPs, and A\( \beta \)40-positive plaque appears in the advanced stage, especially more often in the cored portion of the mature plaque.

In these kits, we use the monoclonal antibodies which specifically detect A\( \beta \). Therefore these kits are designed to be used for the quantitative determination of A\( \beta \) in samples such as tissue culture medium, tissue homogenate, CSF and plasma.

**[Features]**

1. These kits are designed to be used for the quantitative determination of A\( \beta \) in samples such as tissue culture medium, tissue homogenate, CSF and plasma.
2. These kits use the monoclonal antibodies that were developed by Takeda Pharmaceutical Company, Ltd.
   - BAN50: Specifically detects the N-terminal of A\( \beta \) (1-16)
   - BNT77: Specifically detects the A\( \beta \) (11-28) of A\( \beta \)
   - BA27: Specifically detects the C-terminal of A\( \beta \) 40
   - BCOS: Specifically detects the C-terminal of A\( \beta \)42

**[Kit Contents]**

- 1) MAB-coated Microtiter plate [Pkg. Size] (96 tests)
- 2) Standard Solution 2 vials × 2 mL
- 3) Standard Diluent 1 vial × 30 mL
- 4) Wash Solution (20 ×) 1 vial × 50 mL
- 5) HRP-conjugated MAb Solution 1 vial × 12 mL
- 6) TMB Solution 1 vial × 12 mL
- 7) Stop Solution 1 vial × 12 mL
- 8) Plate Seal 3 sheets

**[Principle]**

1. Human \( \beta \) Amyloid (1-40) ELISA Kit wako 2. Human \( \beta \) Amyloid (1-42) ELISA Kit wako 3. Human/Rat \( \beta \) Amyloid (40) ELISA Kit wako 4. Human/Rat \( \beta \) Amyloid (42) ELISA Kit wako

<table>
<thead>
<tr>
<th>Description</th>
<th>Wako Cat. # (Pkg. Size)</th>
<th>human A( \beta ) (1-40)</th>
<th>human A( \beta ) (1-42)</th>
<th>rat(mouse) A( \beta ) (1-42)</th>
<th>rat(mouse) A( \beta ) (1-42)</th>
<th>A( \beta ) (x-40)</th>
<th>A( \beta ) (x-42)</th>
<th>Dynamic Range (pmol/L)</th>
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<tbody>
<tr>
<td>Human ( \beta ) Amyloid (1-40) ELISA Kit Wako [BAN50/BA27(Fab')]</td>
<td>292-62301 (96 tests)</td>
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<td>[●]</td>
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<td>[●]</td>
<td>[●]</td>
<td>[●]</td>
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<tr>
<td>Human ( \beta ) Amyloid (1-40) ELISA Kit Wako II* [BAN50/BA27(Fab')]</td>
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<td>[●]</td>
<td>[●]</td>
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* Improved \( \beta \) Amyloid (1-40) and (x-40)** Detection Kits
** Highly Sensitive \( \beta \) Amyloid (1-42) and (x-42)*** Detection Kits
*** Ab (x-40) and (x-42) are A\( \beta \) peptide modified or cleaved at the N-terminal.

For other products, please visit the Wako Online Catalog  http://www.e-reagent.com
### Standard Curve

#### Human β Amyloid (1-42) ELISA Kit Wako, High-Sensitive

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<th>Mean(n=3) (OD at 450nm)</th>
<th>CV (%)</th>
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#### Human/Rat β Amyloid (42) ELISA Kit Wako, High-Sensitive

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<td>20.0</td>
<td>2.092</td>
<td>1.01</td>
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### Application Data

#### [1] Human and mouse plasma

B. Blood was collected using vacuum blood collection tube containing EDTA-2K. Plasma was separated by centrifugation at 5000 x g for 15 minutes at 4°C and stored at −80°C until used. The plasma sample was diluted 4-fold with Standard Diluent in the Kit and measured.

#### [2] Mouse brain tissue

The hemisphere of 12-month mouse J20 was extracted with 2 mL of Tris Saline and stored frozen at −20°C until used. The brain sample was diluted 2-fold with Standard Diluent in the Kit and measured. A trace quantity of Aβ could be detected not only in the transgenic mice (tg) but in the wildtype mice (wt).

(Data provided by Prof. Iwatsubo and Instructor Hashimoto, Department of Neuropathology and Neuroscience, Graduate School of Pharmaceutical Sciences, University of Tokyo)

### Specificity (n=4)

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<thead>
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<th>Synthetic Peptide</th>
<th>Human Aβ(1-40) Kit II</th>
<th>Human Aβ(1-42) Kit II</th>
<th>Human Aβ(1-42) Kit II</th>
<th>Human/Rat Aβ(42) Kit II</th>
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<td>Human Aβ(1-42)</td>
<td>≤0.1</td>
<td>100.0</td>
<td>≤0.1</td>
<td>100.0</td>
</tr>
<tr>
<td>Human Aβ(1-43)</td>
<td>≤0.1</td>
<td>13.5</td>
<td>≤0.1</td>
<td>12.7</td>
</tr>
<tr>
<td>Rat(Mouse) Aβ(1-40)</td>
<td>0.2</td>
<td>≤0.1</td>
<td>156.0</td>
<td>≤0.1</td>
</tr>
<tr>
<td>Rat(Mouse) Aβ(1-42)</td>
<td>0.3</td>
<td>0.5</td>
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<td>156.0</td>
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### Spike Recovery (n=3)

#### Synthetic Peptide

<table>
<thead>
<tr>
<th>Spiked Amount</th>
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<tbody>
<tr>
<td>5 (pmol/L)</td>
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<tr>
<td>10 (pmol/L)</td>
<td>-</td>
</tr>
<tr>
<td>20 (pmol/L)</td>
<td>-</td>
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</tbody>
</table>
1. Alzheimer's Disease Research

For other products, please visit the Wako Online Catalog  http://www.e-reagent.com

[References]

Histostaining of Alzheimer's Diseased Brain Tissues – Distinctive Histostaining of Aβ40 and Aβ42 plaques

Amyloid β-Protein Immunohistostain Kit

Wako Cat. No. 299-56701  50 tests

Keep at 2-10°C

[Features]
1. Distinctive histostaining of Aβ40 and 42 plaques in Alzheimer’s diseased brain tissues
2. High sensitivity histo-Immune detection of Aβ plaques in tissue sections with low background

[Kit Contents (50 tests)]
1. Blocking Serum 1 bottle × 10 mL
2. Anti Mouse lgG (H+L), Goat, Conjugated 1 bottle × 10 mL
3. ABC Solution (Streptavidin-biotin-peroxidase Complex) 1 bottle × 10 mL
4. Formic Acid (90%) 1 bottle × 15 mL
5. Anti Amyloid β-Protein (1-40), MAb, Clone # BA27 1 bottle × 7 mL
6. Anti Amyloid β-Protein (1-42), MAb, Clone # BV05 1 bottle × 7 mL
7. Trypsin, Cryst 1 bottle × 50 L

[II Kit-conventional kit Correlation]

<table>
<thead>
<tr>
<th>Sample</th>
<th>Human β Amyloid (1-40) ELISA Kit</th>
<th>Human/Rat β Amyloid (40) ELISA Kit</th>
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<tr>
<td></td>
<td>II Kit (F(ab')2-HRP)</td>
<td>the conventional kit (Fab'-HRP)</td>
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<tr>
<td></td>
<td>Human Rat β Amyloid (40) ELISA Kit</td>
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<tr>
<td>plasma A</td>
<td>45.9</td>
<td>56.4</td>
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<tr>
<td>plasma B</td>
<td>49.1</td>
<td>68.1</td>
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<tr>
<td>plasma C</td>
<td>62.4</td>
<td>83.5</td>
</tr>
<tr>
<td>plasma D</td>
<td>40.5</td>
<td>56.1</td>
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<tr>
<td>plasma E</td>
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<tr>
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<td>58.3</td>
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<tr>
<td>plasma G</td>
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<td>76.7</td>
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<tr>
<td>plasma H</td>
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II Kit-conventional kit Correlation

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<tr>
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</table>

Histostaining of senile plaques in the consecutive sections of the brain affected with Alzheimer’s Disease.

Left : Aβ42-staining using Anti Aβ42 Ab (Clone #BC05); Right : Aβ40- staining using Anti Aβ40 Ab (Clone #BA27) (provided by Dr. Iwatsubo, Univ. of Tokyo)
2. Antibodies

for microRNA Research

Antibodies for microRNA Research

Anti Human Ago2, Monoclonal Antibody (Clone No. 4G8)
Wako Cat. No. 011-22033 (50 μL); 015-22031 (100 μL) <for Immunochemistry>
Keep at 2~10°C
☞ Please see the page #22.

Anti Mouse Ago2, Monoclonal Antibody (Clone No. 2D4)
Wako Cat. No. 014-22023 (50 μL); 018-22021 (100 μL) <for Immunochemistry>
Keep at 2~10°C
☞ Please see the page #23.

Study for Cholesteryl Ester Transfer Protein (CETP)

Cholesteryl ester transfer protein (CETP) is one of the lipid transfer proteins, which mediates the transfer of cholesteryl ester (CE), triglyceride (TG) and phospholipids between lipoproteins. CETP facilitates the transfer of CE from high-density lipoprotein (HDL) to very-low-density lipoprotein (VLDL) and low-density lipoprotein (LDL), and also the transfer of TG from VLDL and LDL to HDL. The clinical significance of CETP has been controversial. Wako supplies two kind of CETP monoclonal antibody as well as the ELISA Kit, which is based on the sandwich enzyme immunoassay measures CETP mass.

Anti Human CETP, Monoclonal Antibody (Clone No. CETP-4)*
Wako Cat. No. 010-21241 (100 μg) <for Immunochemistry>
Keep at -80°C
This monoclonal antibody (Clone No. CETP-4) inhibits cholesteryl ester transfer protein (CETP) activity. The 10 μg/mL antibody solution can entirely inhibit the CETP activity in the same quantity of normal serum.

Anti Human CETP, Monoclonal Antibody (Clone No. CM5,a-27)*
Wako Cat. No. 017-21251 (100 μg) <for Immunochemistry>
Keep at -80°C
This monoclonal antibody, specifically recognizes SDS-treated CETP, is applicable to immunostaining on nitrocellulose membrane

*: ELISA kit using both antibodies can be supplied upon request (minimum order: 10 kits).

CUSTOM-ORDERED SUPPLY

Human CETP ELISA Kit
Wako Cat. No. 290-65401 (for 72 tests) <for Immunochemistry>

[Features]
1. Easy determination of human serum CETP
2. High linearity by 4.8 μg/mL
3. 4-hour procedure

[Calibration Curve]

[References]

For other products, please visit the Wako Online Catalog  http://www.e-reagent.com
2. Antibodies

Antibodies against Macrophage/ Microglia-specific Protein Iba1*

* Iba1: ionized calcium binding adapter molecule 1

Anti Iba-1 polyclonal antibody, Rabbit, for Immunocytochemistry
Wako Cat. No. 019-19741 (50 μg (100 μL)) <for Immunocytochemistry>
Keep at -20°C; Working Concentration: 1 – 2 μg/mL (Immunocytochemistry)

Anti Iba-1 polyclonal antibody, Rabbit, for Western Blotting
Wako Cat. No. 016-20001 (50 μg (100 μL)) <for Immunocytochemistry>
Keep at -20°C; Working Concentration: 0.5 ~ 1 μg/mL (Western Blot)

Calcium ions are known to be one of the most important signal mediators in all cells including central nervous system (CNS) cells. Calcium ions exert their signaling activity through association with various calcium binding proteins, many of which are classified into a large protein family, the EF hand protein family.

Iba1 is a 17-kDa EF hand protein that is specifically expressed in macrophages/microglia and is upregulated during the activation of these cells.

Wako distributes rabbit polyclonal antibodies were raised against a synthetic peptide corresponding to the Iba1 carboxy-terminal sequence, which was conserved among human, rat and mouse Iba1 protein sequences. These antibodies are specifically reactive to microglia/macrophages, are appropriate for immuno-double staining of brain tissues and cell culture in combination with monoclonal antibody to GFAP, which specifically reacts to astrocyte.

[Specificity]
Specific to microglia and macrophages, but not cross-reactive with neurons and astrocytes.
Reactive with human, mouse and rat Iba1.

Research for Olfactory Nerve

Anti Olfactory Marker Protein, Goat [Anti OMP]
Wako Cat. No. 544-10001 (100 μL) <for Immunocytochemistry>
Keep at -20°C

Olfactory Marker Protein (OMP) is soluble acid protein expressed in mature olfactory nerve. This goat antiserum is highly specific for mature olfactory neurons and their axons and terminals in tissue sections of many vertebrate species including rodents, humans, marsupials and amphibia.

Working Dilutions:
- Western Blot: ~ 1 : 50,000
- Immunocytochemistry: 1 : 200 (paraffin embedded material) ~ 1 : 50,000 (Vectastain-Elite with fixed floating sections)

Preparation: Goat antiserum to OMP (100μL) is diluted 1:1 with glycerol containing 0.05% sodium azide to facilitate shipment at ambient temperature.

[References]

For other products, please visit the Wako Online Catalog  http://www.e-reagent.com
Study for Human Aging Brain

Anti Phosphorylated α-Synuclein, Monoclonal Antibody (Clone No. pSyn #64)

Wako Cat. No. 014-20281 (50 μL) <for Immunochemistry>

Keep at -20°C

α-Synuclein in Lewy bodies (LBs) which are pathognomonic for Parkinson’s disease (PD) and dementia with Lewy bodies (DLB) contains the phosphorylated at Ser129. We have launched an antibody which specifically reacts with human α-Synuclein with a phosphorylated Ser129 residue and does not react to human α-Synuclein. This antibody is applicable to immunohistochemical and biological studies on the locations of LB-related pathology.

Subclass : Mouse IgG
Specificity : Specific for human α-Synuclein with a phosphorylated Ser129.
No cross-react with human α-Synuclein.
Working Dilution : 1 : 1000 ~ 1 : 10000 (Western blot and Immunochemistry)

[Immunohistochemistry of synucleinopathy lesions and Western blot analysis]

A : Temporal neocortex of DLB brains were immunostained with anti Phosphorylated α-Synuclein. Big arrow (↑) and mini-arrow (↓) indicate LBs and Lewy neurites, respectively.
B : Brainstem LBs in pigmented neurons of the substantia nigra in PD.
C : Western blot analysis of α-synuclein differentially extracted with urea from cerebral cortices of a patient with DLB (D) and a normal control (C) individual probed with monoclonal antibody pSyn#64 (Anti Phosphorylated α-Synuclein). This antibody strongly reacted with the urea-soluble phosphorylated α-synuclein (●) in DLB brains.

[References]

Research for Neurontransmitter and Alzheimer’s disease

Anti Substance-P, Rabbit, affinity purified IgG fraction

Wako Cat. No. 016-13911 (1 mL) <for Immunochemistry>

Keep at -20°C

Substance P is a neuropeptide which is widely distributed in the periphery and the central nervous system, where it is co-localised with other neurotransmitters such as serotonin or dopamine and where it acts as a neuromodulator. Substance P has been proposed to play a role in the antiopathology of asthma, inflammatory bowel disease, emesis, psoriasis, as well as neuropsychiatric disorders including pain syndromes (e.g. migraine and fibromyalgia) and affective disorders, anxiety disorders, schizophrenia and Alzheimer’s disease.

Anti Substance P is isolated from rabbit antiserum against Substance P, and consists of the IgG fraction in 20 mM PBS solution (pH 7.2). The product is treated with Protein A column and affinity purified using Sepharose column.

Appearance: frozen
Protein content: 100 μg IgG/mL (A280nm)
Specificity: Does not cross-react with enkephalin, endorphin, or bradykinin
Antibody titer: 1 : 10,000 (EIA method)

[References]
**Antibodies**

### Autophagy Research

**Anti SQSTM1/A170/p62, Rabbit**

Wako Cat. No. 018-22141 (100 μL) <for Immunochemistry>

**Keep at -20°C**

Sequestosome 1 (SQSTM1) / A170 (mouse) / p62 (human) / ZIP (rat), a ubiquitin-binding protein, expresses oxidative stress-dependently. Abnormality of SQSTM1 leads to bone metabolic disorder, obesity and Type II diabetes. SQSTM1 was reported to bind LC3, which regulates autophagosome formation. The protein has attracted the attention of researchers because it is believed to induce a protein from ubiquitin-proteosome system (UPS) to lysosome-dependent macroautophagy (autophagy) system, which are two major intracellular pathways for protein degradation.

Wako has launched the mouse SQSTM1 (A170) rabbit antiserum, which is applicable to Western blot, immunohistochemistry and immunofluorescence.

**Preparation:** The antiserum is diluted with an equal amount of PBS and absorbed with *E. coli* proteins.

**Immunogen:** recombinant murine SQSTM1 (A170) (AA254-333) containing T7 tag at the N-terminal end and His tag at the carboxy-terminal end

**Specificity:** Specific for mouse and rat SQSTM1 (A170 / ZIP). Slightly reactive with human SQSTM1 (p62).

**Working Dilution:** Western blot 1 : 200 ; Immunohistochemistry 1 : 1,000 ; Immunofluorescence 1 : 1,000

#### APPLICATION-1

**Immunohistochemistry**

- Rat Cerebellar dentate nuclei
- Rat Basal nuclei

Figure: Brain tissues were fixed with 4% paraformaldehyde and embedded with paraffin. The 6μm sections were stained with avidin-biotin-peroxidase method.

**Primary Antibody:** Anti SQSTM1/A170/p62 (Wako Cat. #018-22141) 1 : 1,000

**Secondary Antibody:** Anti rabbit IgG, biotin conjugated

#### APPLICATION-2

**Western blot**

- SQSTM1/A170/p62

Figure: Western blot of cultivated mouse vascular smooth-muscle cell lysate (20μg)

**Antibody:** Anti SQSTM1/A170/p62 (Wako Cat. #018-22141) 1:200

Data was provided from Prof. Ishii, Tsukuba University (Japan)

#### References


### Research for NO

**Anti soluble Guanylate Cyclase (sGC), MAb** (Clone: mAB3221)

Wako Catalog No. 019-17801 (20 μg (40 μL)) <for Immunochemistry>

**Keep at -20°C**

Soluble guanylate cyclase (sGC), a hemoprotein, is the primary nitric oxide (NO) receptor in higher eukaryotes that catalyzes the conversion of guanosine 5’-triphosphate (GTP) to 3,5’-cyclic guanosine monophosphate (cGMP) and pyrophosphate (PPi) in the presence of Mg²⁺. The binding of NO to sGC leads to a several hundred-fold increase in cGMP synthesis.

**Prepared from culture supernatant and prepared in glycine-Tris solution (pH 7.4). Contains no preservatives and stabilizers.**

**Isotype:** IgG1

**Specifically reacts with rat, bovine and human sGC, and strengthens in the reactivity on activation of sGC by NO, probably, due to the conformational changes of the enzyme and its associated antibody-antigen complex.**

**Working Dilution:**

- Westernblot 1 : 5,000 ; Immunofluorescence 1 : 250

#### Reference

**Antibodies**

**Anti soluble Guanylate Cyclase (sGC), MAb, NO insensitive** (Clone: mAB28131)

Wako Catalog No. 017-18201 (20 μg (40 μL)) <for Immunochemistry>
Keep at -20°C

Prepared from culture supernatant and prepared in glycine-Tris solution (pH 7.4). Contains no preservatives and stabilizers.

Isotype: IgG1
Specifically reacts with rat, bovine and human β-subunit of sGC, but not strengthened in the reactivity on activation of sGC by NO.

Working Dilution:
- Western blot 1: 5,000; Immunofluorescence 1: 250

**Antibodies against Vesicular Glutamate Transporters**

**Anti Rat VGLUT-1, Rabbit**

Wako Catalog No. 010-19771 (50 μg (100 μL)) <for Immunochemistry>
Keep at -20°C

L-Glutamate is an excitatory chemical transmitter that plays an essential role in neuronal plasticity, behavior, learning and memory in the central nervous system. On the other hand, VGLUTs play an essential role in glutamate signal output through vesicular storage of L-glutamate. Three kinds of VGLUTs have been identified so far. Recent studies have demonstrated that VGLUTs are also expressed in peripheral cells such as stomach, intestines, pancreas and testes. In particular, the discovery that glutamate is co-localized with glucagon in secretory granules in cells of islets of Langerhans has been noted for new mechanism of blood glucose control. Anti Rat VGLUT, Rabbit can detect glutamatergic central nerves, peripheral nerves and nonneural cells.

Both antibodies are applicable to immunocytochemistry, immunoelectron microscopy and Western blotting.

**Anti Rat VGLUT-2, Rabbit**

Wako Catalog No. 017-19781 (50 μg (100 μL)) <for Immunochemistry>
Keep at -20°C

VGLUT2 is localized in the acrosome.

**Application [1]: Immunofluorescence**

- VGLUT1 is co-localized with glucagon in cells of islets of Langerhans.
- VGLUT2 is co-localized with glucagon in L-cells in the mucosa of ileum.

**Application [2]: Immunoelectron microscopy**

- Double immunoelectron microscopy in cells of islets of Langerhans. Glucagon (5nm) and VGLUT2 (15nm) (arrowheads) are co-localized with secretory granules.

Photo by Dr. Mitsuko Hayashi (Yale Univ.)

**References**

3. Apoptosis Research

Apoptosis Detection Kit by TUNEL method

Apoptosis in situ Detection Kit *wako*

Wako Cat. No. 298-60201 (40 tests) <for Apoptosis Research>

Keep at -20°C

The kit is based on TUNEL [Terminal deoxynucleotidyl Transferase(TdT)-mediated dUTP nick end labeling] procedure, that is the addition of fluorescein-dUTP to 3’-terminals of apoptotically fragmented DNA with TdT followed by immunochemical detection using anti-fluorescein antibody conjugated with horseradish peroxidase (POD) and DAB as a substrate.

[Features]
1. Rapid detection can be performed. The whole process from the de-paraffinizing step to the microscopic examination can be completed in about 2 hours.
2. Complicated preparations of various reagents are not needed. The kit contains the essential reagents required for detection of apoptosis.
3. The kit shows a clear positive image with low background.

[Applicable samples]
- paraffin-embedded tissue sections
- frozen tissue sections
- neutralized formalin-fixed culture cells

[TUNEL Staining]

Cultured cell CHO-K1: after Apoptosis induction (CPZ treatment) (x 400)
Nuclei: Methyl Green Staining

Rat small intestine (x 400)
Nuclei: Methyl Green Staining

Rat testicle: DAB Intensifying Staining (x 200)

Human T cell lymphoma: HE Staining (x 200)

Human B cell lymphoma: HE Staining (x 200)

Human gastric cancer (x 200)
Nuclei: Methyl Green Staining

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<tr>
<th>Kit Contents</th>
<th>(approx. 1cm² x 40 reactions)</th>
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<tr>
<td>Protein Digestion Enzyme</td>
<td>1 vial x 1 mL</td>
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<tr>
<td>TdT</td>
<td>1 vial x 40 μL</td>
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<tr>
<td>TdT Substrate Solution</td>
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<tr>
<td>100 x POD-Conjugated Antibody</td>
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<tr>
<td>DAB Solution</td>
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<td>DNase I</td>
<td>1 vial x 4 μL</td>
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<th>Package Size</th>
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<tr>
<td>Apoptosis in situ Detection Kit <em>wako</em></td>
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<td>40 tests</td>
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Related Products

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<td>293-56601 (4 x 1 cm²)</td>
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<tr>
<td>Lemosol® (&lt;limonene-based solvent as a xylene substitute&gt;)</td>
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<tr>
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<td>120-04411 (1 L)</td>
<td>Protect from Light</td>
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<tr>
<td>Softmount (&lt;a mounting reagent containing Lemosol® A&gt;)</td>
<td>199-11311 (250 mL)</td>
<td>Protect from Light</td>
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<tr>
<td>1 x PBS (-) Powder (0.01 mol/L, pH 7.2~7.4)</td>
<td>162-19321 (for 1 L x 20)</td>
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<td>Methyl Green Solution (0.5 w/v%)</td>
<td>138-12701 (100 mL)</td>
<td>Keep at 2~10°C</td>
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</table>

For other products, please visit the Wako Online Catalog  http://www.e-reagent.com
for semi-quantifying apoptotic cells in culture cells using TUNEL

**Apoptosis Screening Kit Wako**

Wako Cat. No. 296-60001 (96 tests)
Keep at -20°C <for apoptosis research>

Apoptosis Screening Kit Wako is designed to semi-quantify apoptotic cells in culture cells grown in a microtiter plate using the TUNEL (Terminal deoxynucleotidyl Transferase (TdT)-mediated dUTP nick en labeling) procedure. Fluorescein-labeled fragmented DNA by the TUNEL procedure is immunochemically quantified by horseradish peroxidase (POD)-conjugated antibody and a chromogenic substrate on a microplate. This kit provides all the essential reagents for the assay.

**[Features]**
1. Rapid detection can be performed in 3 hours.
2. Tedious preparations of various reagents are not needed.

![Graph](image)

HL-60 (10^7 cells) were seeded in each well and 1μg/mL of actinomycin D was added every one hour. The plate was incubated sequentially and the absorbance was measured. Then, the detection procedure according to the package insert was carried out. Cell death rate was calculated by morphologic observation of the cells under a microscope and the correlation between the rate and the absorbance was examined.

**Apoptosis Inducer**

**Camptothecin, 98.0+/-% (HPLC)**

Wako Cat. No. 038-18191 (100 mg); 034-18193 (500 mg)
Keep at 2~10°C <for Biochemistry>

An alkaloid contained in Nothapodytes foetida and Camptotheca acuminata. It has a quinoline skeleton but, biosynthetically, it is a resembling compound of indole alkaloid.

This product is a reversible inhibitor of DNA topoisomerase I and it binds to topoisomerase-DNA complex leading to stabilization. It exhibits antileukemic and antitumor activities and inhibits activation of HIV-1 by Tat. It exhibits cytotoxicity to tumorigenetic cells but not to non-tumorigenetic cells. It also induces apoptosis in HL-60 cells and mouse thymus cells.

Anticancer irinotecan (Topotecin) is one of its derivatives.

**Apoptosis Inducing Bioprobe**

**Cytotrienin A, from Streptomyces sp.**

039-18241 (100 μg)
Keep at -20°C <for Biochemistry>

A unique bioprobe, cytotrienin A induces apoptosis (or programmed cell death) in human promyelocytic leukemia HL-60 cells at a low concentration (10 ng/mL).

Solubility : Soluble in methanol (0.1 mg/mL)

**[References]**
4. Cell Separation

for Lymphocyte Separation

Iron Powder, from Iron Carbonyl, 99.0+% (Titration)

Wako Cat. No. 098-02222 (25 g) <for Lymphocyte Separation>

Keep at RT

The product is a very fine uniform iron powder used for macrophage elimination.

CAS No. 7439-89-6

Appearance: Grayish black, powder
Particle Size: approx. 6 μm
Bulk specific gravity: 3~4 g/mL
Solubility: Soluble in dil.HCl, dil.H₂SO₄, dil.HNO₃ with producing hydrogen gas.

[References]
(2) Thierfelder, S.: "A METHOD FOR THE ISOLATION OF HUMAN LYMPHOCYTES", Vox Sang, 9, 447-54 (1964)

for T-cell Separation

Nylon Fiber

Wako Cat. No. 146-04231 (5 × 2 g); 142-04233 (100 g) <for T-cell Separation>

Keep at RT

Appearance: White~slightly pale yellow, fibrous
Divalent cations (as Ca²⁺): 10+ μg/g
Solubility: Soluble in dil.HCl, dil.H₂SO₄, dil.HNO₃, with producing hydrogen gas.

A simple method for the preparation of highly enriched, unselected and unaltered populations of T cells has been described by Julius. By packing nylon wool fiber into columns, efficient isolation of T cells from antisera without B cell contamination is possible. Wako has improved upon the same method, offering nylon wool fiber which is free of all toxic substances, in addition, pre-washed, pre-packaged nylon wool fiber comes ready to use in sterilized 10cc-columns, saving hours of preparation. Prepackaged columns also eliminate variability in column packing, insuring consistent cell elution rates.

Nylon Fiber Columns

Nylon Fiber Column T

Wako Cat. No. 147-06721 (10 syringes × 0.5 g) <for mouse T-cell Separation>

Keep at RT

Nylon Fiber Column T (L-Type)

Wako Cat. No. 143-07041 (10 syringes × 1 g) <for human, rabbit and rat T-cell Separation>

Keep at RT

We offer two sizes of Nylon Fiber Column T; Nylon Fiber Column T is for separation of T-cells of mouse lymphocytes and the L-type is for that of a large amount of suspended solution, especially rat splenic lymphocytes, and peripheral blood lymphocytes of rabbit and human.

[Features]
1. Simple procedure.
2. High reproducibility.
3. Sterilized.
4. Using a high quality nylon fiber.

Cell Recovery:
13 ~ 25% (mouse) when Nylon Fiber Column T was used.
25 ~ 35% (rat), 20 - 30% (human) and 25 ~ 30% (rabbit) when Nylon Fiber Column T (L-Type) was used.

B-cell contamination: Less than 15%

[Reference]
Green Chemiluminescent probe for superoxide anions

Green Chemiluminescent CD
Wako Catalog No. 075-05111 (1 mg)
Keep at -20°C

Green Chemiluminescent CD is a highly sensitive chemiluminescence probe, which was developed by Dr. Teranishi of Mie University, Japan. This probe reacts with superoxide anion and produces luminescence at long wavelengths. Therefore the luminescence remains nearly unaffected by biomaterials. Additionally, this probe is more sensitive than other probes which produce luminescence at long wavelengths.

[Features]
1. High luminescence intensity
2. Luminescence at long wavelengths (530 nm)

[Solubility]
Soluble to hot methanol : H₂O (1:1) containing 0.1% TFA.

[Reference]

L-012 Sodium Salt, 98.0+% (HPLC)
[8-Amino-5-chloro-7-phenylpyrido [3,4-d] pyridazine-1,4-(2H,3H) dione sodium salt]
Wako Catalog No. 120-04891 (100mg)
Keep at -20°C, Solid

L-012, which is a highly sensitive chemiluminescent (CHL) probe, is more active than luminol. L-012 reacts with various types of reactive oxygen species generated by activated neutrophils in human blood and oral cavity, and from peritoneal cavity of the rat. This product can be applied to any other EIA that uses horseradish peroxidase to improve sensitivity.

[References]
6. Fluorescent Probes

<table>
<thead>
<tr>
<th>Cell Permeability</th>
<th>Description (Grade) Physical data, etc.</th>
<th>Wako Cat. No. (Pkg. Size)</th>
<th>Note</th>
</tr>
</thead>
<tbody>
<tr>
<td>×</td>
<td>BES-Thio</td>
<td>C&lt;sub&gt;28&lt;/sub&gt;H&lt;sub&gt;18&lt;/sub&gt;N&lt;sub&gt;2&lt;/sub&gt;O&lt;sub&gt;11&lt;/sub&gt;S = 590.51</td>
<td>025-15481 (1 mg)</td>
</tr>
<tr>
<td>O</td>
<td>&lt;Detection of cell-derived H&lt;sub&gt;2&lt;/sub&gt;O&lt;sub&gt;2&lt;/sub&gt;&gt;</td>
<td>BES-H&lt;sub&gt;2&lt;/sub&gt;O&lt;sub&gt;2&lt;/sub&gt;-Ac&lt;sup&gt;−&lt;/sup&gt;</td>
<td>029-15381 (1 mg)</td>
</tr>
<tr>
<td>×</td>
<td>BES-H&lt;sub&gt;2&lt;/sub&gt;O&lt;sub&gt;2&lt;/sub&gt; (cell-impermeant)</td>
<td>C&lt;sub&gt;26&lt;/sub&gt;H&lt;sub&gt;9&lt;/sub&gt;O&lt;sub&gt;7&lt;/sub&gt;F&lt;sub&gt;7&lt;/sub&gt;S = 598.40</td>
<td>021-16201 (1 mg)</td>
</tr>
<tr>
<td>O</td>
<td>&lt;Detection of cell-derived superoxide&gt;</td>
<td>BES-So-AM&lt;sup&gt;−&lt;/sup&gt;</td>
<td>021-15601 (1 mg)</td>
</tr>
<tr>
<td>×</td>
<td>BES-So (cell-impermeant)</td>
<td>C&lt;sub&gt;28&lt;/sub&gt;H&lt;sub&gt;15&lt;/sub&gt;O&lt;sub&gt;11&lt;/sub&gt;NF&lt;sub&gt;4&lt;/sub&gt;S = 649.48</td>
<td>028-16211 (1 mg)</td>
</tr>
</tbody>
</table>

Fluorescent Bioprobe for Visualization of PSA in Living Cells

DAMPAQ-22 | C<sub>31</sub>H<sub>32</sub>O<sub>4</sub>N<sub>4</sub>S = 556.68 | 049-30761 (2 mg) | Keep at RT See the page #32. |

Mg<sup>2+</sup>-Selective Fluoroionophore

KMG-20-AM | C<sub>19</sub>H<sub>19</sub>No<sub>6</sub> = 357.36 | 110-00711 (1 mg) | Keep at -20°C See the page #18. |

Thiol / Selenol selective fluorescent probe

**BES-Thio**

Several chemiluminescent and fluorescent reagents are known as a thiol group detection reagent, and used for detection of thiol groups or measurement of cholinesterase activity.

Most of these reagents have a low hydrophilicity, so separate reaction steps, enzyme and detection reactions, are required.

BES-Thio has a high hydrophilicity and can be used in aqueous solution. This feature makes it easy to measure the enzyme activity such as cholinesterase using acetylthiocholine or butyrylthiocholine as a substrate.

Furthermore, by pH adjustment, BES-Thio can also detect selenol groups, which sulfur (S) in thiol group is substituted by selenium (Se), and can be used for selenoprotein detection reagent.

**[Features]**

1. High hydrophilicity
2. Respond to thiol at pH 7.4
3. Respond to selenol at pH 5.8

**[References]**

**6. Fluorescent Probes**

### Highly Selective Fluorescent Probe for Hydrogen Peroxide

**BES-H$_2$O$_2$-Ac (cell-permeant), BES-H$_2$O$_2$ (cell-impermeant)**

Reactive oxygen species (ROS) such as superoxide (O$_2^-$), hydrogen peroxide (H$_2$O$_2$), and the hydroxyl radical (HO$^-$) are important mediators of pathological processes in various diseases. 2',7'-Dichlorofluorescein (DCFH) and its diacetyl derivative have been widely used as fluorescent probes for measuring cell-derived H$_2$O$_2$, but these compounds suffer from the major drawback that they are poorly selective toward H$_2$O$_2$.

Wako has launched two kinds of BES-H$_2$O$_2$, which are probes for cell-derived H$_2$O$_2$ and cell-impermeant H$_2$O$_2$ with high selectivity. BES-H$_2$O$_2$-Ac is applicable to clarifying cell response as well as dynamic function of H$_2$O$_2$ with diseases.

**Features**

1. Highly selectivity toward H$_2$O$_2$
2. Applicable to Molecular Imaging

---

**Fluorescent images**

**Phase-contrast images**

---

**References**

**Superoxide selective fluorescent probe**

**BES-So-AM (cell-permeant), BES-So (cell-impermeant)**

Since superoxide (O$_2^-$) is a reactive oxygen having weak cytotoxicity, it is getting attention as a molecule positioned on the uppermost stream side of various reactive oxygen species. O$_2^-$ is detected based on different chemiluminescence and fluorescence methods. Among these detection methods, hydroethidine is commonly used but existing probes including hydroethidine are pointed out to have low selectivity for O$_2^-$. BES-So shows a fluorescence by non-redox-reaction dependent mechanism, and indicates high selectivity for O$_2^-$.

**Features of BES-So-AM**

After cellular uptake, deacetoxymethyl compound by the action of cellular esterase responds to O$_2^-$.  

**References**


---

**Fluorescent images**

- **O$_2^-$-produced stimulation (1)**
- **No O$_2^-$-produced stimulation (2)**
- **O$_2^-$-produced stimulation with O$_2^-$-scavenger (3)**

**Phase-contrast images**

Fluorescent images of Jurkat T cells with BES-So-AM and the same-field phase-contrast images

Jurkat T cells were cultured in a medium with 33μM BES-So-AM at 37°C for 1 hour. Then, one group (1) was cultured in the medium with 4 mM butyric acid (O$_2^-$-produced stimulation), whereas the other group (2) in a medium without butyric acid (no O$_2^-$-produced stimulation) at 37°C for 1 hour. A group (3) was cultured in a medium with 33μM BES-So-AM and Tiron (super-oxide scavenger), and then 5 mM butyric acid was added in the medium.

(Courtesy: Professor Hatsuo Maeda, PhD, School of Pharmacy, Hyogo University of Health Sciences)
Fluorescent Bioprobes for Visualization of Puromycin-Sensitive Aminopeptidase (PSA) in Living Cells

**DAMPAQ-22**

Wako Catalog No. 049-30761 (2 mg) <for Cellbiology>
Keep at RT
☞ See the page #32.

**Mg^{2+}-selective Fluoroionophore**

**KMG-20-AM**

Wako Catalog No. 110-00711 1 mg
Wako Catalog No. 116-00713 5 mg
Keep at -20°C

Dynamic distribution of Mg^{2+} in living cells can be done due to selective recognition of Mg^{2+} by KMG-20-AM. KMG-20-AM is much less reactive to Ca^{2+} than Mg^{2+}. KMG-20-AM enables accurate measurement of Mg^{2+} because it has very much low affinity to Ca^{2+} compared to Mg^{2+}.

Appearance: Brown, powder

Assay (HPLC): 95+% 

[Features]

1. Mg^{2+}-imaging without interference of Ca^{2+}
2. Precise observation of Mg^{2+} distribution by Fluorescent Microscopy
3. Direct observation of Mg^{2+} ion dynamics in living cells

**Fluorescent imaging**

**Figure:** Dynamics of Mg^{2+} probe (KMG-20-AM) in neuron by addition of K^{+}.

**Figure:** Absorption spectra (A) and fluorescence spectra (B) of 10.0 μM KMG-20-AM before and after the addition of MgCl_{2} at 37°C in 10.0 mM HEPES, 120.0 mM KCl, 20.0 mM NaCl (pH 7.2). [MgCl_{2}]=0, 0.1, 0.5, 1, 2, 5, 10, 20, 50, 100, 200, 500 mM. Excitation at 445 nm for the fluorescence measurements.

**Figure:** Responses of fluorescence intensity of KMG-20-AM and Magnesium Green for Ca^{2+}. Arrow indicates the timing of 10 μM CaCl_{2} addition ([Ca^{2+}] increased from 140 to 850 nm).

**References**

**microRNA “Specific” Purification Kit**

**microRNA Isolation Kit, Human Ago2**
Wako Catalog No. 292-66701 (10 Reactions) <for Genetic Research>

microRNA Isolation Kit, Human Ago2 is patent pending. (11, 30, 2007)

**microRNA Isolation Kit, Mouse Ago2**
Wako Catalog No. 292-67301 (10 Reactions) <for Genetic Research>

microRNA Isolation Kit, Mouse Ago2 is patent pending. (11, 30, 2007)

microRNA Isolation Kit, Ago2 can prepare high purity fractions of microRNA, which are bound with Argonaute2 (Ago2) protein, based on immunoprecipitation method by using a high affinity monoclonal antibody against Ago2.

The purified microRNA fraction will contain very little contaminated degradation fragments of rRNA and tRNA.

These kits will highly improve the microRNA cloning efficiency compared with conventional microRNA purification method.

**[Comparison with conventional method]**

<table>
<thead>
<tr>
<th>Conventional method</th>
<th>Wako’s 1-step Method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total RNA extraction</td>
<td>necessary</td>
</tr>
<tr>
<td>small RNA (≤ 200nt) purification</td>
<td>unnecessary</td>
</tr>
<tr>
<td>Denaturing PAGE</td>
<td></td>
</tr>
<tr>
<td>Gel extraction from denaturating PAGE</td>
<td></td>
</tr>
<tr>
<td>Gel purification from denaturating PAGE</td>
<td></td>
</tr>
</tbody>
</table>

**[Outline of procedure]**

**[Simple procedure]**

**[Features]**
1. High purification performance of microRNA
2. Ago2 Specific
3. Little contamination of other RNAs
4. High efficiency of microRNA cloning

**[Kit Contents (10 reactions)]**

1) Anti Ago2 Antibody Beads Solution 500μL × 1 vial
2) Cell Lysis Solution 500μL × 1 vial
3) Elution Solution 500μL × 1 vial
4) Ethachinmate 30μL × 1 vial
5) 3 mol/L Sodium Acetate 400μL × 1 vial

[Cloning of purified microRNA from HeLa cells]

High efficiency of microRNA cloning by using this kit followed by using microRNA Cloning Kit Wako.
microRNA Isolation Kit, Human Ago2
Wako Catalog No. 292-66701 (10 Reactions)

- Lane M: Molecular weight marker
- Lane 1: Single strand RNA (22nt) 1ng
- Lane 2: HeLa
- Lane 3: HepG2 (Human)
- Lane 4: HEK293
- Lane 5: P388D1 (Mouse)

Figure 1: Purification of microRNA fractions by using microRNA Isolation Kit, Human Ago2. The purified microRNA fractions from HeLa cells were specifically detected by Urea-PAGE. Cell number is approximately 5\times10^6.

---

microRNA Isolation Kit, Mouse Ago2
Wako Catalog No. 292-67301 (10 Reactions)

[Purification of microRNA fractions from several rodent cell lines]

- Lane M: RNA Molecular weight marker
- Std: Single strand RNA (22nt) 1ng
- Lane 1: HeLa (Human) (Negative control)
- Lane 2: P388D1 (Mouse)
- Lane 3: CHO-K1 (Chinese Hamster)
- Lane 4: PC-12 (Flat)

Figure 2: Urea-PAGE pattern of purified RNA by using microRNA Isolation Kit, Mouse Ago2 (Wako catalog #292-67301). The purified microRNA fractions from cultured rodent cell lines (P388D1, CHO-K1, PC-12) were detected by silver stain. Cell number of each cell line is approximately 5 \times 10^6. The applied volume per lane is half of isolated sample by this kit.

---

[Cloning of purified microRNA from P388D1 cells]

High efficiency of microRNA cloning by the combination use of microRNA Cloning Kit Wako (Wako Cat. No. 290-66501)

Table 1. The contents of cloned microRNA.

<table>
<thead>
<tr>
<th>microRNA</th>
<th>The number of clone</th>
</tr>
</thead>
<tbody>
<tr>
<td>mmu-miR-92a</td>
<td>40</td>
</tr>
<tr>
<td>mmu-miR-23a</td>
<td>21</td>
</tr>
<tr>
<td>mmu-miR-25</td>
<td>5</td>
</tr>
<tr>
<td>mmu-miR-315</td>
<td>5</td>
</tr>
<tr>
<td>mmu-miR-31</td>
<td>2</td>
</tr>
<tr>
<td>mmu-miR-23b</td>
<td>2</td>
</tr>
<tr>
<td>mmu-miR-22</td>
<td>2</td>
</tr>
<tr>
<td>mmu-miR-21</td>
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</tr>
<tr>
<td>mmu-miR-7d</td>
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<tr>
<td>mmu-miR-632</td>
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<tr>
<td>mmu-miR-423</td>
<td>1</td>
</tr>
<tr>
<td>mmu-miR-132</td>
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</tr>
<tr>
<td>mmu-miR-18a</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
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</tr>
</tbody>
</table>

Figure 3: Cloning efficiency of microRNA from P388D1 cell lysate. The presence ratio of microRNA was more than 90%. Others indicated cDNAs which were listed in miRBase of other organism species. Unknowns indicated cDNAs which were found in genome sequence, but not listed in miRBase. The contents of cloned microRNA are indicated on Table 1.

High cloning efficiency

[Procedure of microRNA cloning]

1) The microRNA fraction was prepared by microRNA Isolation Kit, Mouse Ago2.
2) The CDNA encoding microRNA was synthesized by microRNA Cloning Kit Wako and inserted it into T-vector.
3) The 96 transformants of E. coli were randomly selected from selection LB agar medium.
4) Inserted cDNA sequences were determined by DNA sequencer and collected sequences by using data base of Sanger miRBase.

For other products, please visit the Wako Online Catalog http://www.e-reagent.com
“High Efficiency” microRNA Cloning Kit
microRNA Cloning Kit Wako
Wako Catalog No. 290-66501 (8 Reactions) <for Genetic Research>

The microRNA Cloning Kit Wako can prepare the cDNA encoding microRNA. The cloning procedure will be completed within 1.5 days after preparation of microRNA fraction. This kit is supported by shrimp alkaline phosphatase (SAP), thermostable single strand DNA ligase (which is selling separately: Wako Cat. #292-65101 (500 units); #298-65103 (200 units)), and original modified adaptors.

The cloning efficiency using this kit is improved higher than that of the conventional methods, which used bacterial alkaline phosphatase and T4 RNA ligase.

[Features]
1. High cloning efficiency
2. Cloning of secondary structured microRNA
3. High reproducibility of microRNA Cloning

[Procedure of microRNA cloning]
1) Preparation of total RNA from HeLa cells (1×10^7 cells) by ISOGEN (Nippon Gene #315-02504, 10mL).
2) Preparation of small RNA fraction, less than 200nt, from total RNA by microRNA Isolation Kit (Bio Chain Institute Inc. catalog #KS341025).
3) Separation of microRNA fraction by denaturing PAGE.
4) Collection of the gels of 20~23nt region after electrophoresis.
5) Cloning by using microRNA Cloning Kit Wako (Wako catalog #290-66501).
6) Construction of the plasmids harboring cDNA encoding microRNA and transformation of E. coli.
7) Random selection of the 96 transformed E. coli from selection LB agar medium.
8) Determination and verification of the cDNA sequences by using Sanger miRBase.

<table>
<thead>
<tr>
<th>microRNA species</th>
<th>The number of clone</th>
</tr>
</thead>
<tbody>
<tr>
<td>hsa-miR-23a</td>
<td>38</td>
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<td>hsa-miR-92a</td>
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<td>hsa-miR-23b</td>
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<tr>
<td>hsa-miR-19b</td>
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<tr>
<td>hsa-miR-21</td>
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<tr>
<td>hsa-miR-210</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>70</td>
</tr>
</tbody>
</table>

MicroRNA cloning and verification:

[Outline of procedure]
- Dephosphorylation
- Adaptor ligation
- Reverse transcription
- PCR
- Identification of cloned microRNA species

Cloning efficiency:
- Cloning efficiency of microRNA from HeLa cell lysate was more than 70%.
- Others indicate that isolated cDNA sequences were not matched miRBase. Unknowns indicate that isolated cDNA sequences were not matched human genome sequence. The contents of cloned microRNA species are indicated on Table 1.
Argonaute2 (Ago2) was isolated as one of the main components of RISC (RNA-induced silencing complex). Ago2 captures siRNA and microRNA which are working as a guide molecule for interaction with target mRNAs in RNAi pathway. In this pathway, Ago2 catalyzes the nicking of target mRNAs and binding between RISC and target mRNAs. This monoclonal antibody is not only used for western blot and immunocytochemistry (ICC), but also immunoprecipitation (IP) of hAgo2.

**[Features]**
1. For IP, ICC, Western Blot
2. Specific reactivity with human Ago2 protein
3. For purification of RNA captured by RISC

**Concentration (protein):** Indicated on the label.
**Formulation:** 0.09% Sodium Azide, 10% Glycerol with 1x TBS, pH7.4.
**Subclass:** IgG1
**Antigen:** Recombinant human Ago2
**Storage:** 2~10°C in the dark. Avoid the freeze and thaw.

**[References]**

**APPLICATION DATA**

**Immunoprecipitation of hAgo2 protein from HeLa cell line**

**Immunocytochemistry of hAgo2 protein of HeLa cell line**

(Provided by Haruhiko Siomi, PhD and Mikiko Siomi, PhD)

**Figure 1** (A) Western blot of hAgo2 protein from HeLa cell lysate. The band of hAgo2 protein was detected in approximately 100kDa. Working dilution of this product was 1/100 dilution. Cell number was 5×10⁶ cells.

(B) Immunoprecipitation of hAgo2 protein from HeLa cell lysate by using Gamma-bind beads immobilized with this antibody. The band of hAgo2 protein was detected in approximately 100kDa by using silver staining. Cell number was 5×10⁶ cells.

**Figure 3** Immunoprecipitation of hAgo2 protein from human cultured cell lines (HeLa, HepG2, HEK293, THP-1) and mouse cultured cell line (P388D1) by using 20μL 10% Protein G slurry immobilized with 10μg this antibody. The bands of hAgo2 protein were detected in approximately 100kDa by using silver staining and western blot. Cell number was 5×10⁶ cells.

**Figure 4** Purification of microRNA fraction from immunoprecipitated hAgo2 protein. The purified microRNA fraction from human cultured cell lines (HeLa, HepG2, HEK293, THP-1) were specifically detected by Urea-PAGE. Cell number of each cell line is 5×10⁶ cells. The applied volume per lane is half of 10μL of final solution prepared with an IP.
Argonaute2 (Ago2) was isolated as one of the main components of RISC (RNA-induced silencing complex). Ago2 captures siRNA and microRNA which are working as a guide molecule for interaction with target mRNAs in RNAi pathway. In this pathway, Ago2 catalyzes the nicking of target mRNAs and binding between RISC and target mRNAs. This monoclonal antibody is not only used for western blot and immunocytochemistry (ICC), but also immunoprecipitation (IP) of mAgo2.

[Features]
1. For IP, ICC, Western Blot
2. Cross reactivity with Ago2 protein of rat & hamster
3. For purification of RNA captured by RISC

Concentration (protein): Indicated on the label.
Formulation: 0.05% Sodium Azide, 10% Glycerol with 1 × TBS, pH7.4.
Subclass: IgG1
Antigen: Synthesized peptide of N terminal mouse Ago2.
Storage: 2~10°C in the dark. Avoid the freeze and thaw.

[Reference]

APPLICATION DATA

Immunoprecipitation of mAgo2 protein from P388D1 cell line

Figure 1. Immunoprecipitation of mAgo2 protein from P388D1 cell line by using 20μL 10% Protein G slurry immobilized with 5μg this antibody (2D4). The band of endogenous mAgo2 protein was detected in approximately 100kDa by using silver staining and western blot. The 1/1,000 diluted this antibody was used as the 1st antibody for western blot. Cell number was 5 ×10^6 cells.

Figure 2. Immunoprecipitation of mAgo2 protein from NIH-3T3 cell line by using 20μL 10% Protein G slurry immobilized with 5μg this antibody (2D4). The band of endogenous mAgo2 protein was detected in approximately 100kDa by using silver staining and western blot. The 1/1,000 diluted this antibody was used as the 1st antibody for western blot. Cell number was 5 ×10^6 cells.

Figure 3. Immunoprecipitation of Ago2 protein from NIH-3T3 (Mouse), SCC-131(Rat) and CHO(Hamster) cell line by using 20μL 10% Protein G slurry immobilized with 5μg this antibody (2D4). The band of endogenous Ago2 protein was detected in approximately 100kDa by using silver staining and western blot. The 1/1,000 diluted this antibody was used as the 1st antibody for western blot. Cell number was 5 ×10^6 cells.

Figure 4. Immunoprecipitation of Ago2 protein from NIH-3T3(Mouse), SCC-131(Rat) and CHO(Hamster) cell line by using 20μL 10% Protein G slurry immobilized with 5μg this antibody (2D4). The band of endogenous Ago2 protein was detected in approximately 100kDa by using silver staining and western blot. The 1/1,000 diluted this antibody was used as the 1st antibody for western blot. Cell number was 5 ×10^6 cells.

Application Working Dilution
Western Blot 1 : 200 - 1 : 1,000
Immunoprecipitation 5~10μg / IP
Immunocytochemistry 1 : 100 - 1 : 500

<table>
<thead>
<tr>
<th>Organisms</th>
<th>Mouse</th>
<th>Hamster</th>
<th>Rat</th>
<th>Human</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell</td>
<td>P388D1</td>
<td>CHO</td>
<td>SCC-131</td>
<td>NCI-H460</td>
</tr>
<tr>
<td>Western Blot</td>
<td>O</td>
<td>O</td>
<td>O</td>
<td>X</td>
</tr>
<tr>
<td>Immunoprecipitation</td>
<td>O</td>
<td>O</td>
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<td>X</td>
</tr>
<tr>
<td>Immunocytochemistry</td>
<td>O (NIH-3T3)</td>
<td>NT</td>
<td>NT</td>
<td>X</td>
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<tr>
<td>microRNA Purification</td>
<td>O (P388D1)</td>
<td>O</td>
<td>O</td>
<td>X</td>
</tr>
</tbody>
</table>

NT : Not Tested.
8. Physiological Active Substances

17-AAG [17-(Allylamino)-17-desmethoxygeldanamycin; Allylaminogeldanamycin]
Wako Cat. No. 012-20101 (1 mg) <for Cellbiology>
Keep at -20°C
Potential, less toxic derivative of geldanamycin. Inhibits the essential ATPase activity of HSP90, apoptosis with antitumor activity.

- Appearance: Red ~ dark purple, crystalline powder ~ powder
- Solubility: Soluble in DMSO (10mg/mL) or methanol (10mg/mL)

[References]

ADP Ribosyltransferase C3, from Clostridium botulinum
Wako Cat. No. 011-14441 (10 μg) <for Biochemistry>
Keep at -20°C
Neurotoxin: Botulinum neurotoxin C3 with no toxicity

- Source: Clostridium botulinum
- Appearance: Lyophilized
- Solubility: Soluble in ethanol, methanol and acetone.
- Activity: Approximately 1 pmol/mg P2/μg C3
- Unit Definition: An amount of ADP-ribose required for the formation of substrate (P2 fraction) 1 mg by 1 μg of ADP-ribosyltransferase C3

[References]

Antibiotics/Folate Metabolism related Substances

Ampicillin
[Anhydrous] Wako Cat. No. 017-10381 (5 g); 015-10382 (25 g) <for Biochemistry>
[Standard; anhydrous] Wako Cat. No. 017-20531 (200 mg) <for HPLC>
[Sodium Salt] Wako Cat. No. 010-10371 (5 g); 016-10373 (10 g); 018-10372 (25 g) <for Biochemistry>
Keep at -20°C

Antibiotics/Folate Metabolism related Substances
An antibiotic which is a synthetic penicillin used in studies on dysentery and urinary tract infections.
It has a broad antibacterial spectrum and is active against Gram-positive and Gram-negative bacteria.
It is also used for checking one of the properties of Ames test strain, i.e. the existence of drug resistance factor plasmid PKM101.

- Appearance: [Anhydrous & the standard] White ~ slightly yellow, powder
- [Na Salt] White, crystalline powder ~ powder
- Assay (HPLC): [Anhydrous] 96.0+%; [Anhydrous standard] 98+%
- Potency (calculated on the dehydrous basis): [Sodium Salt] 850+μg/mg
- Solubility: [Anhydrous] Soluble in MeOH or water. Slightly soluble in EtOH.
- Practically insoluble in ether.
- [Na Salt] Freely soluble in water. Sparingly soluble in EtOH.
8. Physiological Active Substances

**Anisomycin**, 96.0+ % (HPLC)
Wako Cat. No. 017-16861 (10 mg); 013-16863 (50 mg); 011-16864 (250 mg) <for Biochemistry>
Keep at -20°C

Antibiotic. Activator of p38 and MAP kinases. Synergistic with growth factors and phorbol esters to superinduce cFos and cJun, by acting as a potent signalling agonist. Induces apoptosis in the human monoblastoid cell line. Used in the eradication of bean mildew. Inhibits other pathogenic fungi in plants.

Source: Isolated from *Streptomyces griseolus*
Appearance: White ~ slightly yellow, crystalline powder ~ powder
Solubility: Soluble in DMSO (25mg/mL), 100% ethanol, methanol or ethyl acetate (10mg/mL)

**Aristeromycin**
Wako Cat. No. 015-09691 (5 mg) <for Biochemistry>
Keep at -20°C

The product is an antibiotic which inhibits the growth of plant pathogens such as Xanthomonas oryzae and Piricuraria oryzae. Carbocyclic nucleoside antibiotic. It inhibits the synthesis of AMP in mammalian cells and S-adenosylhomocysteine hydrolase activity.

Source: *Streptomyces citricolor*
Appearance: White ~ grayish white, powder or mass
Solubility: Soluble in water and N, N-dimethylformamide

**Bafilomycin A1**, 95.0+% (HPLC)
Wako Cat. No. 023-11641 (100μg); 029-11643 (1 mg) <for Biochemistry>
Keep at -20°C

Bafilomycin A1, isolated from *Streptomyces* sp., is a macrolide antibiotic which inhibits vacuolar-type H⁺-ATPases with a high degree of specificity. Bafilomycin A1 thus serves as an ideal tool for distinguishing among the different types of ATPases that exist in eukaryotic cells. Among them, vacuolar-type ATPase (V-ATPases) are widely distributed in the central vacuolar system, consisting of endosomes, trans-Golgi network, lysosomes, and secretion granules. While V-ATPases are extremely sensitive to the antibiotic and are affected by nanomolar concentrations, F-ATPases (F1F0-type) are unaffected, and P-ATPases (E1E2-type) are only moderately affected at the same concentration.

Source: *Streptomyces griseus*
Appearance: White, Crystals ~ powder or film
Solubility: Soluble in DMSO, ethanol and ethyl acetate. Slightly soluble in water.

[References]

**Bleomycin Hydrochloride**
Wako Cat. No. 028-07801 (10 mg) <for Biochemistry>
Keep at 2~10°C

This is an anticancer antibiotic acting on various cancers. It inhibits DNA synthesis in *Escherichia coli*, HeLa cells and Ehrlich cancer cells and, moreover, inhibits their cell divisions at a low concentration.

Appearance: White ~ pale yellow, powder or small mass
Potency: 1,400 ~ 2,000 μg/mg (calculated on the dried basis)

For other products, please visit the Wako Online Catalog  http://www.e-reagent.com
8. Physiological Active Substances

**Bleomycin Sulfate**
Wako Cat. No. 027-15941 (10 mg); 023-15943 (50 mg)  
< for Pharmacology Research >
Keep at 2~10°C

Anticancer antibiotic
Appearance: White ~ pale brown, Crystalline powder ~ powder
Potency: 1,500+ IU/mg (calculated on the dried basis)

**α-Bungarotoxin**
Wako Cat. No. 026-07961 (1 mg)  < for Biochemistry >
Keep at 2~10°C

Neurotoxin which binds irreversibly to motor endplate of acetylcholine receptors; prevents opening of nicotinic receptor-associated ion channels

Source: *Bungarus multicinctus*
Appearance: White, lyophilized

[Reference]

**Marine Natural Product - Protein Phosphatase Inhibitor**
**Calyculin A**, 95.0+ % (HPLC)
Wako Cat. No. 038-14453 (10 μg); 032-14451 (100 μg)  < for Biochemistry >
Keep at -20°C

Inhibitor of protein phosphatases types 1 and 2A; marine toxin, potent tumor promotor.

Source: *Disodermia calyx*
Appearance: White ~ pale yellow, Crystals ~ powder or film.
Solubility: Soluble in methanol or ethanol. Insoluble in water.

[Reference]

**Capsaicin**
Wako Cat. No. 039-15963 (20 mg); 033-15961 (100 mg), 90.0+ % (HPLC)  
< for Biochemistry >
Wako Cat. No. 034-11351 (100 mg); 030-11353 (1 g), 60.0+ % (HPLC)  
< for Wako 1st Grade >
Wako Cat. No. 039-18981 (20 mg), 99.0+ % (HPLC)  
< for Crude Drugs Determination >
Keep at 2~10°C

An active component contained in capsicum (CAPSICI FRUCTUS). It is a neurotoxin acting on the peptide-containing nervous system and is used as a pharmacological means to elucidate the functional roles of neuropeptides.
Appearance: White ~ pale brown, Crystals ~ powder or mass
Solubility: <for Biochemistry> Soluble in ethanol. Slightly soluble in water.
**Physiological Active Substances**

---

**Carbenicillin Sodium Salt**
- **Wako Cat. No.** 032-18954 (1 g); 038-18951 (5 g); 034-18953 (10 g) *< for Cellbiology>*
- **Wako Cat. No.** 030-17671 (1 g); 036-17673 (5 g); 034-17674 (10 g) *< for Biochemistry>*
- **Keep at** 2~10°C

Penicillin antibiotic acting on Gram-negative bacteria; bacterial cell-wall synthesis inhibitor

<table>
<thead>
<tr>
<th>Appearance: White ~ slightly greenish yellow, crystalline powder ~ powder</th>
</tr>
</thead>
<tbody>
<tr>
<td>Potency: 770+ µg/mg (calculated on the dried basis)</td>
</tr>
<tr>
<td>Solubility: Soluble in water (0.5 g/50 mL)</td>
</tr>
</tbody>
</table>

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**Chloramphenicol**
- **Wako Cat. No.** 032-19451 (5 g); 030-19452 (25 g); 038-19453 (100 g) *< for Molecular Biology, DNase and RNase tested>*
- **Wako Cat. No.** 036-10571 (5 g); 034-10572 (25 g); 032-10573 (100 g) *< for Biochemistry>*
- **Wako Cat. No.** 037-19641 (200 mg) *< 98.0+ % (HPLC), Standard for HPLC>*
- **Keep at** 2~10°C

Antibiotics
The product inhibits protein synthesis in bacteria thus suppressing their proliferation (bacteriostatic effect). It exhibits a strong activity especially against Gram-negative bacilli such as Salmonellae and rickettsia such as epidemic louse-borne typhus and chigger. This is an analogue of nitrobenzene. Although it has a wide antimicrobial spectrum, it is used only in cases where other antibiotics are ineffective or in critical conditions due to its strong adverse reactions.

<table>
<thead>
<tr>
<th>Appearance: White ~ slightly yellow, crystals ~ crystalline powder</th>
</tr>
</thead>
<tbody>
<tr>
<td>Solubility: Freely soluble in methanol and ethanol. Slightly soluble in water and ether.</td>
</tr>
</tbody>
</table>

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**Ciclosporin A, 97.0+ % (HPLC)**
- **Wako Cat. No.** 035-16303 (100 mg); 039-16301 (200 mg) *< for Biochemistry>*
- **Wako Cat. No.** 031-18963 (50 mg); 035-18961 (200 mg) *< for Cellbiology>*
- **Keep at** 2~10°C

<Pharmacologic and Physiologic Research><Autacoid>
Like hormones, autacoids exhibit strong bioactivity in small amounts, but unlike hormones they have no particular production organ, and they refer to biologically active substances expressing strong physiological effects immediately at the site of production. Chemically, they are classified into amines (such as histamine and serotonin), peptides (such as bradykinin and angiotensin) and fatty acids (such as prostaglandin). The product is a typical immunosuppressant used for prevention of graft rejection after organ transplantation. It is a cyclic polypeptide consisting of 11 amino acids and inhibits secretion of interleukin 2 from helper T cells, which promote immune reaction, and thus inhibits immunity.

<table>
<thead>
<tr>
<th>Appearance: White, crystalline powder ~ powder or mass</th>
</tr>
</thead>
</table>

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**Vacuolar H⁺-ATPase Inhibitor**

**Concanamycin A, 90.0+ % (HPLC)**
- **Wako Cat. No.** 036-16034 (25 µg); 032-16031 (100 µg) *< for Biochemistry>*
- **Keep at** -20°C

Concanamycin A is a specific inhibitor of V-ATPases. Isolated from an antifungal biotic of the 18-membered macrolide lactones, Concanamycin A inhibits blastogenesis of cultured cells stimulated by Concanavalin A (Con-A). While Concanamycin A is structurally and pharmacologically similar to Bafilomycin A1. It exhibits about 10 times stronger inhibition than that by Bafilomycin A1.

<table>
<thead>
<tr>
<th>Appearance: White, crystals ~ powder</th>
</tr>
</thead>
<tbody>
<tr>
<td>Solubility: Freely soluble in chloroform, methanol, ethanol, aceton, ethyl acetate and DMSO.</td>
</tr>
</tbody>
</table>

---

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8. Physiological Active Substances

Akt (Protein Kinase B; PKB) inhibitor

Deguelin
Wako Cat. No. 047-29211 (5 mg) <for Cellbiology>
Keep at 2~10°C

It inhibits proliferation of cells in the GM-2 stage of cell cycle. It induces apoptosis in the precancerous and canerated cell lines. The derris root, a legume growing naturally in the South Seas and the tropics, contains a highly potent ingredient lethal to fish and insects. This is an analogue of the active component, rotenone.

Appearance: Slightly pale yellow ~ yellow, crystalline powder ~ powder
Solubility: Soluble in acetone, dichloromethane, acetonitrile and DMSO.

[References]

Marine Natural Products – Protein Phosphate Inhibitor

Dinophysistoxin-I [DTX-I]
Wako Cat. No. 042-28661 (100 μg) <for Biochemistry>
Keep at 2~10°C

Dinophysistoxin, isolated from Halichondria okadai, is a diarrhetic shellfish toxin with 35-methyl okadaic acid. Dinophysistoxin-I is a potent Non-TPA* type tumor promoter and specifically inhibits protein phosphatases.

*TPA: 12-o-Tetradecanoyl-phorbol-13-acetate

Source: Halichondria okadai
Appearance: film
Solubility: Soluble in methanol, ether, acetone, ethyl acetate and chloroform.

[Reference]

HSP60 Inhibitor

ETB [Epolactaene Tertiary Butyl Ester] (mixture of isomers)
Wako Cat. No. 051-07671 (200 μg) <for Cellbiology>
Keep at -20°C

Wako has launched a new inhibitor which were discovered by Dr. Hiroyuki Osada, Antibiotics laboratory of Institute of Physical and Chemical Research (RIKEN) under license from RIKEN. This product is a derivative of epolactaene isolated from Penicillium. It has a more potent cytostatic effect on human neuroblastoma cells SH-SY 5Y than that of epolactaene, and induces apoptosis. Furthermore, it has been revealed that ETB induces apoptosis in human T-lymphoma cells Jurkat. Recently, HSP60 was identified as one of ETB binding proteins. ETB binds to HSP60 to inhibit chaperone activity.

Appearance: White ~ slightly pale brown, crystalline powder ~ powder
Solubility: Soluble in methanol (1 vial is dissolved with 0.2 mL of methanol).

[Reference]
Bacterial Protein Synthesis Inhibitor
Gentamicin Sulfate

Wako Cat. No. 073-02971 (250 mg); 079-02973 (1 g); 077-02974 (5 g); 071-02972 (25 g) <for Biochemistry>
Wako Cat. No. 078-04981 (250 mg); 074-04983 (1 g); 072-04984 (5 g) <for Molecular Biology>

Keep at 2~10°C

This product is an antibiotic that has an antimicrobial activity against gram positive and negative bacteria. It inhibits the initiation of protein synthesis of bacteria by acting as a ribosome to induce misreading of codons. It is a mixture of gentamicin C1, C2, and C1a.

As a reagent for molecular biology, it has been confirmed for DNase and RNase activities.

Source: Micromonospora purpurea
Appearance: White ~ slightly pale brown, powder
Potency: 590+ µg/mg (calculated on the dried basis)
Solubility: Freely soluble in water.
Practically insoluble in ethanol

Highly Selective γ-Glutamyl transpeptidase (GGT) Inhibitor
GGsTop™

Wako Cat. No. 075-05471 (10 mg) <for Cellbiology>

Keep at -20°C

GGsTop™ is a highly selective γ-Glutamyl Transpeptidase (GGT) inhibitor. While acivicin [AT-125], which is widely used as a GGT inhibitor also inhibits asparagine synthetase (GA family), GGsTop™ does not inhibit the asparagine synthetase.

**[Features]**

1. High specificity to GGT
   Acivicin deactivates more than 90% of 100µM of E. coli asparagine synthetase for 2 hours. On the other hand, GGsTop™ did not deactivates even the 10mM enzyme.
2. High inhibitory activity to human GGT
   
<table>
<thead>
<tr>
<th></th>
<th>to human GGT</th>
<th>to E. coli GGT</th>
</tr>
</thead>
<tbody>
<tr>
<td>GGsTop™</td>
<td>51</td>
<td>170</td>
</tr>
<tr>
<td>Acivicin [AT-125]</td>
<td>0.40</td>
<td>4,200</td>
</tr>
</tbody>
</table>

3. Low toxicity
   On acute toxicity test, there is no toxicity with intravenously-infused GGsTop™ (30mg/kg). On the other hand, acivicin has severe toxicity to the CNS.
4. Chemically stable
   The reconstituted neutral or acid aqueous solution such as 0.1% TFA solution and 0.1N HCl solution is stable for 1 month at room temperature for NMR analysis.

**[Reference]**

A synthetic analog of Coenzyme Q10
Idebenone

Wako Cat. No. 096-05001 (100 mg) <for Biochemistry>

Keep at 2~10°C

Idebenone is known to act on the central nervous system (CNS) and ameliorate cerebral apoplexy, cerebral ischemia with affective disorder, tetraplegia, and impaired passive avoidance response.

Appearance: Yellowish red ~ orange, crystals ~ crystalline powder or mass
Solubility: Soluble in ethanol.

**[Reference]**

For other products, please visit the Wako Online Catalog  http://www.e-reagent.com
Neurotoxin – Glutamate Receptor Selective Agonist

Joro Spider Toxin [JSTX-3], 98.0+ % (HPLC)
Wako Cat. No. 104-00051 (0.1 mg) <for Biochemistry>
Keep at 2~10°C
JSTX-3 is derived from the venom of Nephila clavata and consist of three active principles of similar chemical structure and function. Each of these components has been found to be a potent antagonist of neurotransmitter receptors. Wako offers JSTX-3, a chemical of low molecular weight which selectively inhibits excitatory synaptic transmission by blocking quisqualate-sensitive L-glutamate receptors. The high degree of specificity JSTX-3 exhibits makes it an especially valuable tool for the study of neurological disorders and for the research of excitatory neurotransmitter mechanisms.

Appearance: Lyophilized
Solubility: Soluble in water

Neurotoxin – Glutamate Receptor Selective Agonist

Kainic Acid n-Hydrate, 98.0+ % (HPLC)
Wako Cat. No. 118-00751 (10 mg) <for Biochemistry>
Keep at 2~10°C
It is an amino acid with glutamate skeleton isolated from a red algae, Digenea (Corsican weed, Digenea simplex) known as an ascaricide. This product is one of selective agonist for kainate-type glutamate receptor and has a potent CNS stimulating effect. It is used for studies on the signal transduction system via kainate cascade, neuronal apoptosis, ALS (amyotrophic lateral sclerosis), and pathological mechanism of Alzheimer's disease.

Potent Synthesis Inhibitor - Aminoglycoside Antibiotic

Kanamycin Sulfate
Wako Cat. No. 117-00341 (1 g); 113-00343 (5 g); 115-00342 (25 g); 111-00344 (100 g) <for Biochemistry>
Wako Cat. No. 113-00701 (1 g); 119-00703 (5 g); 117-00704 (100 g) <for Cell Culture>
Keep at 2~10°C
Appearance: White ~ slightly pale yellow, crystals ~ powder or mass
Potency: 600+ μg/mg (calculated on the dried basis)
Solubility: Freely soluble in water. Practically insoluble in ethanol and ether.

Neurotoxin - NMDA-Glutamate Receptor Antagonist

(+)-MK 801 Maleate [Dizocilpine Maleate], 98.0+ % (HPLC)
Wako Cat. No. 134-15461 (10 mg); 130-15463 (50 mg) <for Cellbiology>
Keep at 2~10°C
Acts by binding to a site located within the NMDA associated ion channel. It is a non-competitive antagonist showing selectivity for NMDA-type glutamate receptor. It binds to the pore of the ion channel, which is opened by the binding of ligands, and acts as an open-channel blocker.
Appearance: White ~ nearly white, crystals ~ powder

Marine Toxin – Actin Inhibitor

Mycalolide B, 98.0+ % (HPLC)
Wako Cat. No. 132-12081 (100 μg) <for Biochemistry>
Keep at -20°C
Inhibits actin polymerization. Mycalolide B depolymerizes F-actin by nibbling and forms a 1:1 complex with G-actin.
Source: Mycale sp.
Appearance: Clear film
Solubility: Soluble in methanol, ethanol and DMSO.
**Physiological Active Substances**

### Polymethoxy Flavonoids derived from Shekwasha

**Nobiletin, 95.0+ % (HPLC)**

Wako Cat. No. 149-07521 (10 mg) <for Biochemistry>

**Tangeretin, 95.0+ % (HPLC)**

Wako Cat. No. 208-15671 (10 mg) <for Biochemistry>

Keep at -20°C

Nobiletin and tangeretin are polymethoxy flavonoids contained in the juice of Shekwasha (Citrus depressa Hayata), a citrus fruit. These flavonoids are receiving attention for a variety of beneficial effects such as reducing elevation of blood pressure and plasma glucose levels.

- **Nobiletin**
  - Appearance: White ~ slightly pale yellow, crystalline powder ~ powder or mass
  - Solubility: Soluble in ethanol, methanol and acetone.

- **Tangeretin**
  - Appearance: White ~ nearly white, crystalline powder ~ powder
  - Solubility: Soluble in ethanol and methanol. Insoluble in water.

### Protein phosphatase inhibitor

**Okadaic Acid**

- **[Okadaic Acid]** Wako Cat. No. 150-01653 (25 μg); 154-01651 (100 μg) <for Biochemistry>
- **[Ammonium Salt]** Wako Cat. No. 156-02211 (100 μg); 152-02213 (500 μg) <for Biochemistry>

Keep at 2~10°C

Okadaic acid is a causative agent of diarrhetic shellfish poisoning and a potent and specific inhibitor of protein phosphatases 1 (PP1) and 2A (PP2A) that is isolated from the sponge Halichondria Okadai.

- Appearance: [Okadaic Acid] Lyophilized, film; [Ammonium Salt] Lyophilized
- Assay (HPLC): [Okadaic Acid] 80.0+%
- Solubility: [Okadaic Acid] Soluble in DMF, DMSO, Chloroform-methanol. Slightly soluble in water and n-hexane.
- [Ammonium Salt] 100 μg/100 μL (water)

### Neurotoxin; Na⁺ Channel Agonist

**Palytoxin, 90.0+ % (HPLC)**

Wako Cat. No. 161-15131 (100 μg) <for Biochemistry>

Keep at 2~10°C

Palytoxin is a potent marine toxin which acts as a strong hemolysin, histamine releaser, inhibitor of sodium-potassium ATPase, and cation ionophore.

- Appearance: Film (invisible due to very small quantity)
- Solubility: Soluble in pyridine, DMSO and water. Slightly soluble in ethanol and methanol. Insoluble in chloroform, ether and acetone.

### References

**Puromycin-Sensitive Aminopeptidase (PSA) Inhibitor**

**PAQ-22** [3-(2,6-diethylphenyl)-2,4(1H,3H)-quinazolinedione]

Wako Cat. No. 165-23581 (10 mg) <for Cellbiology>

Keep at RT

**Fluorescent Bioprobe for Visualization of PSA in Living Cells**

**DAMPAQ-22**

Wako Cat. No. 049-30761 (2 mg) <for Cellbiology>

Keep at RT

Puromycin-sensitive aminopeptidase (PSA), which is a neutral aminopeptidase with a substrate specificity similar to that of aminopeptidase N (APN), is distributed mainly in the brain and neurons. The physical roles/functions of PSA remain unclear. Wako has launched non-peptide, small-molecular, non-competitive PSA Inhibitor, PAQ-22 and the structurally modified fluorescent bioprobe, DAMPAQ-22. The cellular localization of PSA could be specifically visualized by the use of DAMPAQ-22. These are long-awaited tools for PSA research.

**Table 1. Aminopeptidase-Inhibitory Activity of PAQ-22 and Puromycin**

<table>
<thead>
<tr>
<th></th>
<th>PSA IC50 (μmol/L)</th>
<th>APN IC50 (μmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PAQ-22</td>
<td>3.8</td>
<td>&gt;100</td>
</tr>
<tr>
<td>DAMPAQ-22</td>
<td>4.6</td>
<td>N/A</td>
</tr>
<tr>
<td>Puromycin</td>
<td>0.6</td>
<td>4.8</td>
</tr>
</tbody>
</table>

**[References]**


**New Protein Synthesis Inhibitor - Isoleucyl tRNA Synthesis Enzyme Inhibitor**

**Reveromycin A Sodium Salt**

*Wako Cat. No. 185-02181 (500 μg) <for Cellbiology>*

*Keep at -20°C*

This product is an antibiotic isolated from *Streptomyces*. It targets isoleucyl-tRNA synthetase and inhibits protein synthesis in eukaryotes. It has been investigated for its antitumor and antifungal activities. However, recent studies have revealed that low-dose of reveromycin A induces cell death of activated osteoclasts, which leads to acidic environment. Thus it receives attention as a candidate for the treatment of osteoporosis / multiple myeloma.

This new inhibitor was discovered by Dr. Hiroyuki Osada, Antibiotics laboratory of Institute of Physical and Chemical Research (RIKEN, Japan).

**Source:** *Streptomyces reveromyceticus* SN593  
**Appearance:** Lyophilized

**[Reference]**


---

**Apoptosis Inhibitor**

**RKTS-33**

*Wako Cat. No.182-02191 (200 μg) <for Cellbiology>*

*Keep at -20°C*

This product, which was discovered by Dr. Hiroyuki Osada, Antibiotics Laboratory of Institute of Physical and Chemical Research (RIKEN) under license from RIKEN, is a derivative of epoxycylohexenone isolated from *Paecilomyces*. It has lower toxicity than epoxycylohexenone. Like epoxycylohexenone, it inhibits apoptosis not by inhibition of perforin-dependent pathway by cytotoxic T lymphocytes but by selective inhibition of Fas ligand-dependent pathway alone.

**Source:** *Paecilomyces* sp.  
**Appearance:** Lyophilized  
**Solubility:** Soluble in ethanol

**[Reference]**


---

**Potent Inhibitor of Ca^{2+} Release**

**Ryanodine, from Ryania speciosa, 98.0+% (HPLC)**

*Wako Cat. No. 181-02281 (1 mg); 187-02283 (5 mg) <for Cellbiology>*

*Keep at -20°C*

A Ca^{2+} Channel Inhibitor Ryanodine is an alkaloid isolated from *Ryania speciosa Vahl*. It acts to increase calcium permeability by binding to the sarcoplasmic reticulum calcium channel.

**[References]**


8. Physiological Active Substances

**Calmodulin Inhibitor**

**Stellettamide A Trifluoroacetate, 95.0+ % (HPLC)**

*Wako Cat. No. 193-11831 (100 μg) <for Biochemistry>*

**Keep at -20°C, Lyophilized**

Stellettamide A (ST-A) is a marine toxin isolated from a marine sponge. It is a calmodulin antagonist and inhibits Ca\(^{2+}\)-calmodulin-dependent phosphodiesterase.

Source: *Stelletta* sponge

Solubility: Soluble in methanol (100 μg/100 μL)

**[Reference]**


---

**Protein Phosphatase Inhibitor**

**Tautomycin**

*Wako Cat. No. 209-12041 (100 μg) <for Biochemistry>*

**Keep at -20°C, Lyophilized**

Tautomycin is a highly potent and specific protein phosphatase inhibitor, induced morphological change (bleb-formation) of human myeloid leukemia K562 cells.

The appearance of blebs is inhibited by protein kinase C (PKC) inhibitors. Tautomycin acts as an activator of PKC.

Source: *Streptomycetes spiroverticillatus*

Solubility: Soluble in ethanol. Practically insoluble in water.

**[References]**


8. Physiological Active Substances

**Histone Deacetylase (HDAC) Inhibitor**

**Trichostatin A**, 99.0+ % (HPLC)

*Wako Cat. No. 200-11993 (1 mg); 204-11991 (5 mg) <for Biochemistry>*

*Keep at -20°C*

HDAC plays a central role in chromatin structure formation associated with the nuclear distribution of DNA. There are presently 17 known types of this enzyme in mammals, which are classified into 3 classes. Also, HDAC Class III has been reported to be associated with regulation of aging and life span. HDAC inhibitors show connections with cell division cycles and differentiation, as well as with antitumor activity and apoptosis-inducing activity through the inhibition of the deacetylating activity of HDAC. They can be used for studies on cellular functions involving histone deacetylase.

Trichostatin A (TSA), a *Streptomycyes* product, specifically inhibits the cell cycle of normal rat fibroblasts in the G1 and G2 phases at very low concentrations as reported by Yoshida, *et al.* TSA-induced G2-arrest induces the formation of proliferative tetraploid cells. In addition, nanomolar concentration of TSA has been shown to cause an accumulation of highly acetylated histones *in vivo*, and markedly inhibit the activity of partially purified histone deacetylase *in vitro*.

TSA appears to be a useful product for researching the multiple functions of histone acetylation in regulatory mechanisms of eukaryotic cell proliferation and differentiation.

*Source:* *Streptomycyes Hygroscopicus*

*Solubility:* Soluble in ethanol and acetone. 1 mg/10 mL (methanol)

**References**


**Cell Cycle Inhibitor**

**Tryprostatin A**, from *Xaspergillus fumigatus* BM939

*Wako Cat. No. 203-16961 (500 μg) <for Cellbiology>*

*Keep at -20°C, Lyophilized film*

Tryprostatin A (TPS-A) is an alkaloid antibiotic isolated from *Aspergillus*. It affects the microtubule-associated protein binding site and exhibits antitumor activity by inhibition of cell cycle progression in the M phase specifically.

This product was discovered by Dr. Hiroyuki Osada, Antibiotics laboratory of Institute of Physical and Chemical Research (RIKEN, Japan).

*Reference*


**Membrane-Permeable Inhibitor of IP$_3$ Receptor**

**Xestospongin C**, from *Xestospongia* sp., 90+% (HPLC)

*Wako Cat. No. 244-00721 (100 μg) <for Cellbiology>*

*Keep at -20°C, Lyophilized form in 20 mmol/L HEPES solution (pH 7.3) containing 0.1 % BSA as a stabilizer, packaged under inert gas.*

A selective and membrane-permeable inhibitor of the inositol 1,4,5-triphosphate (IP$_3$) receptor-mediated Ca$^{2+}$ release, isolated from an Okinawan marine sponge *Xestospongia* sp. A potent and highly sensitive inhibitor of IP$_3$ receptor with IC$_{50}$ of 350 nM, which is 30 times lower than that for ryanodine-receptor.

*Reference*

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