DNA Extractor® TIS Kit is specially designed for the extraction of DNA from human and animal parenchyma tissue. The base of this kit is a procedure using Sodium Iodide (NaI) as a chaotropic agent, which allows for a safe extraction without hazardous phenol/chloroform. The procedure using Sodium Iodide is an extraction procedure which causes less oxidation of DNA. Additionally, the DNA Extractor® TIS Kit is able to inhibit the oxidation of DNA by using an Oxidation Inhibitor. It is the most useful DNA extraction kit for the assay of 8-OHdG (8-Hydroxy-2’-deoxyguanosine), which is a marker of oxidative stress.

**Features**
- Useful for detection and determination of 8-OHdG, a marker of oxidative stress.
- Achieve inhibition of the further DNA oxidation by using an oxidation inhibitor
- Suitable for a wide range of applications, which require DNA extraction from human and animal parenchyma tissues.

**DNA extraction from mouse liver and 8-OHdG Assay using the extracted DNA**

DNA was extracted from a mouse liver using DNA Extractor® TIS Kit [Protocol 1] and DNA Extractor® WB Kit (Wako Cat. #291-50502). Both of the obtained DNA were digested to mononucleotides with nuclease P1 using 8-OHdG Assay Preparation Reagent Set (Wako Cat. #292-67801). 8-OHdG was determined by both of High Sensitive 8-OHdG Check (Wako Cat. #307-07921; ELISA method) and HPLC/ECD and obtained values were compared.

**Measurement by ELISA method**

(using High Sensitive 8-OHdG Check)

![Graph showing 8-OHdG concentration (mmol/L) vs. DNA Extractor® WB Kit and DNA Extractor® TIS Kit](image)

<table>
<thead>
<tr>
<th>DNA Extractor® TIS Kit</th>
<th>8-OHdG conc. (mmol/L)</th>
<th>DG conc. (µmol/L)</th>
<th>8-OHdG /10^6dG</th>
</tr>
</thead>
<tbody>
<tr>
<td>DNA Extractor® WB Kit</td>
<td>0.59</td>
<td>692</td>
<td>0.8</td>
</tr>
</tbody>
</table>

(hPLC condition)
- Column: Wakopak® Wakosil® II 5C18 RS (4.6 mm x 150 mm)
- Eluent: 50 mmol/L Sodium Acetate (pH 5.2), 9% Methanol
- Flow rate: 1 mL/min
- Absorbance: UV 260 nm
- ECD Electrolytic Potential: +600 mV

It is known that 8-OHdG is generated by oxidation during the extraction operation of DNA. However, DNA Extractor® TIS Kit could reduce the amount of 8-OHdG generated during operation by more than half compared to the DNA Extractor® WB Kit, by which the oxidation during operation is relatively low. In addition, it was found that the concentration of 8-OHdG in deoxyguanosine was very low. These results indicate that DNA Extractor® TIS Kit has superior performance as a DNA extraction kit for 8-OHdG assay.
8-OHdG Assay Preparation Reagent Set

It is necessary to hydrolyze DNA extracted from tissues when measuring 8-OHdG (8-hydroxy-2′-deoxyguanosine) in tissues. The 8-OHdG Assay Preparation Set contains nuclease, buffer and reagent which are used for the preparation of the assay of 8-OHdG (8-hydroxy-2′deoxyguanosine), a marker for oxidative stress. This kit is able to reduce the variation of the enzyme reaction by addition of each reagent to the sample DNA in accordance with the protocol. Therefore, stable assay results for a marker of oxidative stress can be obtained.

[Protocol]

Tissue 150mg
- 1mL of Lysis Soln. <Homogenization>
  Homogenate in a 2mL-tube 600×G @4°C for 10min.
  ρt
  1mL of Lysis Soln.
  vortex 600×G, 4°C, 10min.
  + 300 μL of Enzyme Reaction Soln.
  + 1 μL of RNase Soln.
  + 3 μL Oxidation Inhibitor
  vortex Incubate @37°C for 10min.
  + 15μL of Protein Digestion Soln.
  Incubate @37°C for 1hr (with vortex every 20min.)
  + 10,000×G @RT for 5min.
  Supernatant/1.5mL-tube
  + 300 μL of NaCl Soln.
  + 600 μL of Alcohol Soln.
  + 10,000×G @RT for 10min.
  DNA Pellet
  + 1mL of 70% EtOH
  10,000×G @RT for 5min,
  DNA Soln.

DNA Pellet
DDW or TE buffer

Kit Contents (50 Reactions)

- Lysis Solution 2 bottles × 75 mL
- Enzyme Reaction Solution 1 bottle × 15 mL
- RNase Solution 1 vial × 50 μL
- Protein Digestion Solution 1 vial × 750 μL
- Oxidation Inhibitor 1 vial × 350 μL
- Sodium Iodide Solution 1 bottle × 15 mL
- Alcohol Solution 1 bottle × 30 mL
- PEG Solution 1 bottle × 20 mL

8-OHdG Assay Preparation Reagent Set

(Wako Catalog No. 292-67801; 50 Reactions)

Kit Contents (50 Reactions)

- Acetic Acid Buffer 1 vial × 950 μL
- Nuclease P1 1 vial × 500 units
- Tris Buffer 1 vial × 1 mL
- Alkaline Phosphatase Solution 1 vial × 50 μL

[Product List]

<table>
<thead>
<tr>
<th>Description</th>
<th>Wako Cat. No.(Pkg. Size)</th>
<th>Application</th>
</tr>
</thead>
<tbody>
<tr>
<td>DNA Extractor® TIS Kit</td>
<td>296-67701 (50 Reactions)</td>
<td>DNA extraction from human/animal parenchyma tissue DNA extraction for 8-OHdG Assay</td>
</tr>
<tr>
<td>8-OHdG Assay Preparation Reagent Set</td>
<td>292-67801 (50 Reactions)</td>
<td>Used for preparation of 8-OHdG assay</td>
</tr>
</tbody>
</table>

[Related Products]

<table>
<thead>
<tr>
<th>Description</th>
<th>Wako Cat. No.(Pkg. Size)</th>
<th>Application</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nuclease P1. <em>Penicillium citrinum</em></td>
<td>145-08221 (500 units)</td>
<td>A kit content of DNA Extractor® TIS Kit</td>
</tr>
<tr>
<td>Keep at 2~10°C; Lyophilized</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Optimum temperature: 70°C; Optimum pH: 5.3 (RNA and heat-denatured DNA)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>High Sensitive 8-OHdG Check</td>
<td>307-07921 (96 tests)</td>
<td>ELISA kit for 8-OHdG determination</td>
</tr>
<tr>
<td>Keep at 2~10°C, manufactured by Nikken Seil Co., Ltd.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wakopak® Wakosil-II 5C18 RS (φ4.6 mm × 150 mm) (D)</td>
<td>238-51471 (1 unit)</td>
<td>HPLC Column</td>
</tr>
<tr>
<td>Wakopak® Wakosil-II 5C18 RS (φ4.6 mm × 150 mm) (W)</td>
<td>234-51473 (1 unit)</td>
<td>HPLC Column</td>
</tr>
</tbody>
</table>

Wako Pure Chemical Industries, Ltd.
http://www.wako-chem.co.jp
1-2, Doshimachi 3-Chome
Chuo-ku, Osaka 540-8605, Japan
Tel: 81-6-6203-3741
Fax: 81-6-6203-1999
Online Catalog: http://www.e-reagent.com

Wako Chemicals USA, Inc.
http://www.wakousa.com
Toll-Free (U.S. only): 1-877-714-1920
Head Office (Richmond, VA):
Tel: 1-804-714-1920 Fax: 1-804-271-7791
Los Angeles Sales Office (Irvine, CA):
Tel: 1-949-679-1700 Fax: 1-949-679-1701
Boston Sales Office (Cambridge, MA):
Tel: 1-617-394-4772 Fax: 1-617-394-6774

Wako Chemicals GmbH
http://www.wakochemie.de
European Office:
Fuggerstraße 12, D-41468
Neuss, Germany
Tel: 49-2131-311-0
Fax: 49-2131-311100