

Wako

# SLP Reagent Set Series



for the detection and measurement of  
peptidoglycan and  $\beta$ -glucan

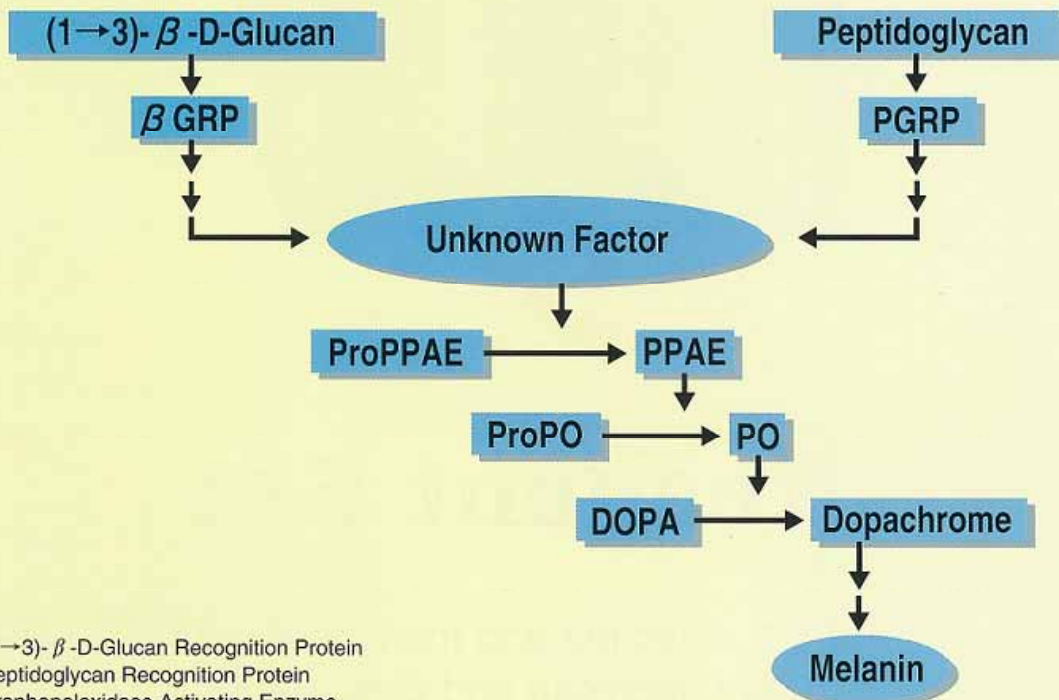
### Introduction

When foreign matter enters into an insect body, melanin is synthesized and the foreign matter is surrounded by the melanin, which is a self-defense system of insects. The melanin formation is considered to be caused by a series of reactions including activation of multiple serine proteases called prophenoloxidase (proPO) cascade.<sup>1</sup> The proPO cascade is activated by peptidoglycan (PG) which is a cell wall component of Gram-positive and Gram-negative bacteria or (1→3)- $\beta$ -glucan (BG) which is a cell wall component of fungi. Silkworm (*Bombyx mori*) body fluid contains the proPO cascade. Wako SLP Reagent Kits are derived from silkworm larvae plasma (SLP), which contain all the factors of the proPO cascade. The SLP Reagent Kits are activated by PG or BG so that microorganisms can be detected by using these reagent kits.

### The proPO cascade

In SLP there are proteins specifically bound to BG and PG, which are named BG recognition protein ( $\beta$ GRP) and PG recognition protein (PGRP), respectively. BG is bound to the  $\beta$ GRP to form the  $\beta$ GRP-BG complex and PG to form the PGRP-PG complex. It has not been found which compounds the  $\beta$ GRP-BG complex or the PGRP-PG complex reacts with. However, when these complexes are formed, precursors of serine protease are activated. One of the precursors is the precursor of proPO activating enzyme (proPPAE). In the cascade reactions the proPPAE hydrolyses proPO and then the final product in the proPO cascade, phenoloxidase (PO) is produced.

## Cascade Reaction of SLP



$\beta$  GRP : (1→3)- $\beta$ -D-Glucan Recognition Protein  
PGRP : Peptidoglycan Recognition Protein  
PPAE : Prophenoloxidase Activating Enzyme  
PO : Phenoloxidase  
DOPA : 3, 4-Dihydroxyphenylalanine

## Principle

When a sample is mixed with the SLP reagent, PG and/or BG in the sample initiate the proPO cascade reactions. The PO produced in the cascade reactions oxidizes the substrate in the SLP reagent, 3,4-dihydroxyphenylalanine (DOPA), to form melanin.

## Features

PG and BG can be assayed with high sensitivity.

No instrument is required when a visual test is performed.

More precise and more sensitive assay can be done with an instrument such as a microplate reader or Wako Toxinometer (a tube reader for the kinetic turbidimetric assay).

PG and BG can be quantitatively assayed by using a microplate reader or Wako Toxinometer .

## Single-Test Reagent with Wako Toxinometer ET-6000

### SLP-HS Single Reagent Set

(Wako Catalog No. 293-58301; 20 tests)

SLP-HS (Silkworm Larvae Plasma-High Sensitive)

20 vials x for 0.2 mL

Sensitivity:

Either 10 pg/mL of PG or 1 pg/mL of BG can be detected with Toxinometer at 30 in 120 min.

Store at 2 ~ 10

SLP-Diluent

20 vials x 1.0 mL

Standard (Digested Peptidoglycan from *S. aureus*)

1 vial x 0.5 mL

## Multi-Test Set

Multi-Test Set

### SLP Reagent Set

(Wako Catalog No. 297-51501; for 3 mL)

For use with a microplate reader or for visual tests, approximately 50 tests, and for use with the Toxinometer, approximately 30 tests can be made. The SLP reagent should be used as soon as possible after reconstitution.

SLP, lyophilized

1 vial for 3 mL

Store at 2 ~ 10

Substrate, lyophilized

1 vial for 3 mL

Store at 2 ~ 10

Diluent

1 vial x 4 mL

Store at 2 ~ 10



## Assay Methods

### Visual Test

Mix the SLP reagent solution with a sample. After a fixed time of incubation, the formation of melanin is visually judged.

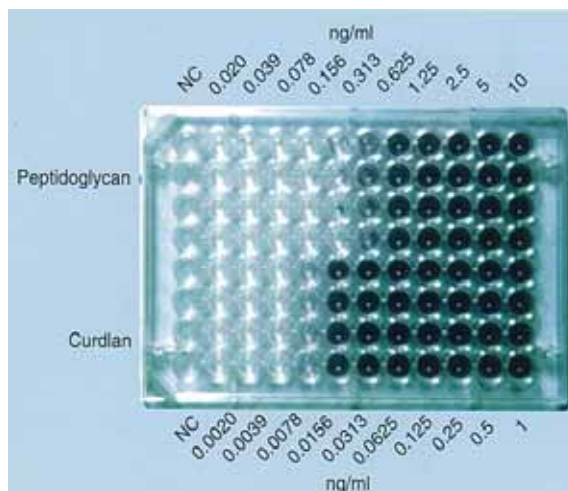
#### Single-Test Reagent (reacted in a tube)

(SLP-HS Single Reagent Set (Wako Cat. #293-58301))



#### Multi-Test Reagent (reacted in a microplate)

(SLP Reagent Set (Wako Cat. No. 297-51501))



## Assay Methods

### Chromogenic measuring method

using Toxinometer ET-6000 or a Microplate Reader

Mix the SLP reagent solution with a sample in a test tube (when Toxinometer used) or a microplate well (when a microplate reader used). During the formation of melanin, transmittance of the reaction mixture decreases. The transmittance ratio and the time taken for the transmittance ratio to reach the threshold value are measured with the Toxinometer ET-6000 or a microplate reader. The concentration of PG or BG in the sample is obtained according



Toxinometer ET-6000 Analysis Module

Toxinometer ET-6000 Part 11 Set

### References

- (1) Ashida, M. and Yamazaki, H. I., Biochemistry of the Phenoloxidase System in Insect: with Special Reference to Its Activation. "Molting and Metamorphosis", ed. By Onishi, E. and Ishizaki, H., *Japan Sci. Soc. Press, Tokyo*.
- (2) Tsuchiya M., Kobayashi, F., Aswahi, N., Yokota, A., and Matsuura, S., Reactivities of Gram-negative bacteria and Gram-positive bacteria with Limulus amebocyte lysate and silworm larvae plasma., *J. Endotoxin Res.*, Vol. 1 suppl., 1, 70 (1994).
- (3) Tsuchiya, M., Asahi, N., Suzuoki, F., Ashida, M., and Matsuura, S., Detection of peptidoglycan and  $\beta$ -glucan with silkworm larvae plasma test., *FEMS Immunol. Med. Microbiol.*, 15, 129-134 (1996).

### Products

Description	Wako Catalog No. (Pkg. Size)	Note
<b>SLP Reagent Set</b>	<b>297-51501 (25 tests)</b>	Keep at 2 ~ 10
Kit Contents: (1) SLP, lyophilized (1 vial x for 3 mL) / (2) Substrate, lyophilized (1 vial for 3 mL) / (3) Diluent (1 vial x 4 mL)		
<b>SLP-HS Single Reagent Set</b>	<b>293-58301 (20 tests)</b>	Keep at 2 ~ 10
Kit Contents: (1) SLP-HS (Silkworm Larvae Plasma-High Sensitive) (20 vials x for 0.2 mL) / (2) SLP-Diluent (20 vials x 1.0 mL) / (3) Standard (Digested Peptidoglycan from <i>S. aureus</i> ) (1 vial x 0.5 mL)		
Peptidoglycan, from <i>Micrococcus luteus</i>	162-18101 (2 mL (1 $\mu$ g/mL))	Keep at 2 ~ 10
Curdlan [(1-3)- $\beta$ -D-glucan]	030-09903 (1 g)	Keep at 2 ~ 10
Toxinometer ET-6000/ U Part 11 Set	293-33509 (1 unit)	100-120 $\pm$ 10% VAC 50/60 Hz
Toxinometer ET-6000/ U Anaalysis Module	297-33529 (1 unit)	
Toxinometer ET-6000/E Part 11 Set	290-33519 (1 unit)	220-240 $\pm$ 10% VAC 50/60 Hz
Toxinometer ET-6000/E Anaalysis Module	294-33539 (1 unit)	
Limulus Test Tube-S with Aluminum Cap	292-32751 (80 tubes)	12 x 75 mm tube with alminum cap; Endotoxin free
Limulus Test Tube-S	293-26551 (80 tubes)	12 x 75 mm tube; Endotoxin free
Aluminum Cap-S	293-28251 (100 caps)	14.7 x 18 mm; Endotoxin free.
BioCleanTip Wako® 1000	294-31351 (100 tips)	70 ~ 72 mm length; for 1000 $\mu$ L; Endotoxin and BG free
BioCleanTip Wako® 200	290-31451 (100 tips)	50 ~ 52 mm length; for 200 $\mu$ L; Endotoxin and BG free
BioCleanTip Wako® Extend S	298-32851 (100 tips)	94 ~ 96 mm length; for 200 $\mu$ L; Endotoxin and BG free

Listed products are intended for laboratory research use only, and not to be used for drug, food or human use. / Please visit our online catalog to search for other products from Wako; [www.e-reagent.com](http://www.e-reagent.com) / This leaflet may contain products that cannot be exported to your country due to regulations.

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