FUJIFILM
Value from Innovation

Wako

SLP-HS SINGLE REAGENT SET II

PEPTIDOGLYCAN AND β-GLUCAN DETECTION

QUANTITATIVE ANALYSIS OF PYROGENS THROUGH KINETIC COLORIMETRIC ASSAY

+ Suitable for Toxinometer® ET-7000
+ Compliant with the FDA Title 21 CFR Part 11

INTRODUCTION

The hemolymph of silkworm *Bombyx mori* contains a self-defense mechanism referred to as the "prophenoloxidase cascade system (Pro-PO)", which is triggered by peptidoglycan (PG) and (1→3)-β-D-glucan (BDG), resulting into activation of prophenoloxidase (PO) and production of toxic intermediates against invading pathogens.

Although the exact mechanism is not yet fully elucidated, it has been postulated that different serine proteases are involved in this process, eventually leading to cleavage of the inactive Pro-PO to the active PO and formation of melanin.

The SLP reagent is a lyophilized product prepared under sterile conditions from the silkworm hemolymph, containing all factors involved in the Pro-PO cascade system. Upon activation by PG and BDG, melanization occurs over two steps, through oxidation of L-DOPA (*L*-3,4-Dihydroxyphenylalanine) into Dopachrome and subsequent conversion of latter one into black melanin pigment, thus, resulting in color change of the solution. Since PG is found in all bacterial cell walls and BDG in most fungi, the SLP-reagent constitutes a highly sensitive and broadband method for detection of microbial contamination.

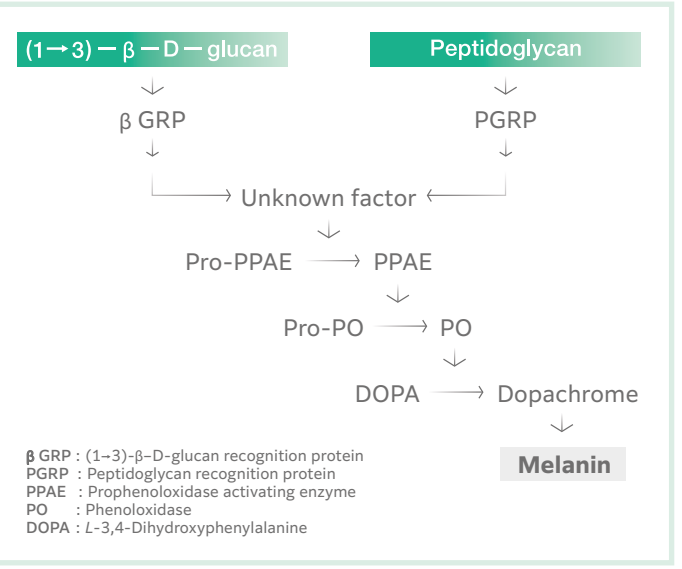


Fig. 1: The activation mechanism of SLP by PG and BDB
Binding of PG and/or BDG to their respective recognition proteins (PGRP or GRP) initiates the Pro-PO reaction cascade, eventually leading to conversion of Prophenoloxidase into active Phenoloxidase. The activated enzyme then catalyzes oxidation of L DOPA into Dopachrome, which is then converted into black melanin pigment.

INTENDED USE

QUANTITATIVE DETERMINATION OF PEPTIDOGLYCAN AND β-GLUCAN IN PARENTAL SOLUTIONS AND ACTIVE PHARMACEUTICAL INGREDIENTS

TEST PRINCIPLE

The SLP reagent strongly reacts with PG and BDG, inducing formation of black melanin pigment, which can be monitored, either through visual detection, or quantitatively by measuring the absorbance at 650 nm.

For latter application the analysis can be performed by using in combination with the Toxinometer® ET-7000.

The quantification is achieved through monitoring of melanin formation by measuring the activation time (Ta or onset time) of the reaction, i.e. the point in time at which the absorbance reaches a predetermined threshold. By correlating the measured value to a previously generated calibration curve with a PG standard, the absolute concentration of PG and BDG can be determined with a sensitivity in the lower pg.ml^{-1} range.

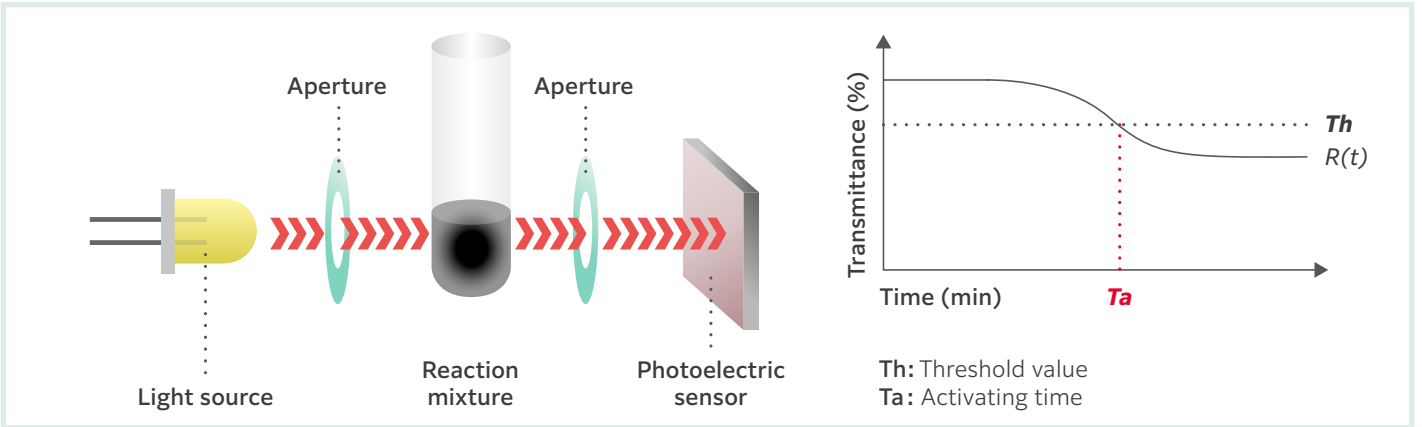


Fig. 2: Optical system for quantitative analysis of pyrogens (PG and/or BDG) through kinetic colorimetric assay and reaction time course.

INSTRUMENTS

CODE	PRODUCT	PACKAGE
293-36061	Toxinometer® ET-7000	Toxinometer® ET-7000 (1 unit)
299-36161	Toximaster® QC8 Part11 PC Set	Toximaster® QC8 Part11 Software Personal computer (1unit) System validation documents
292-36271	Toxinometer® ET-7000 240 V power cord (for the area of 200~240 V <E Type>)	240 V power cord for ET-7000

REAGENTS AND RELATED PRODUCTS

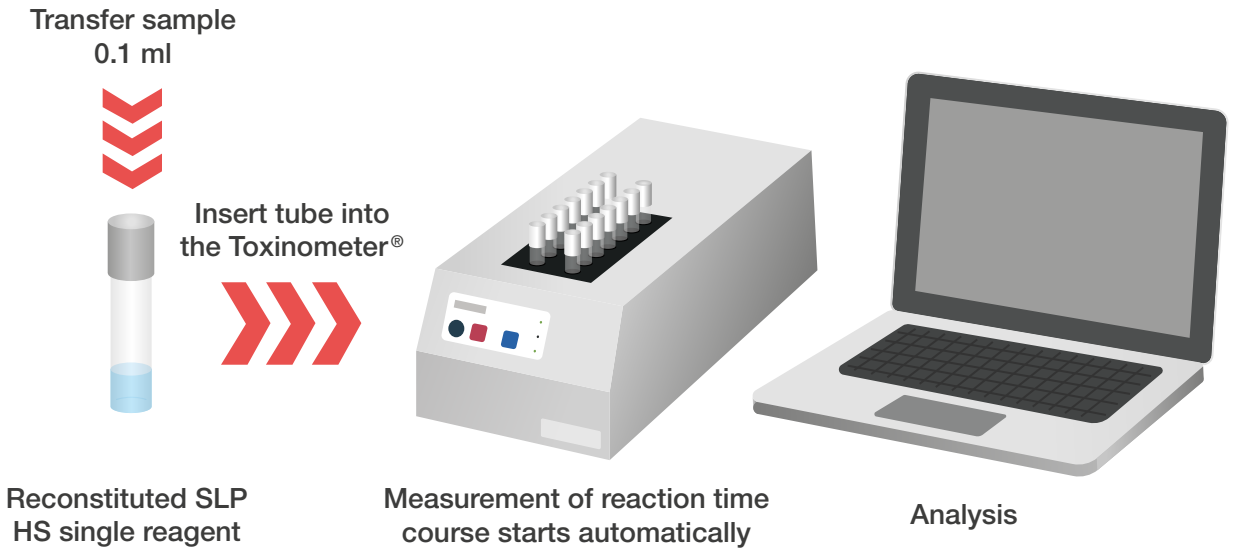
CODE	PRODUCT	PACKAGE
296-81001	SLP-HS Single Reagent Set II	**SLP-HS Reagent II (lyophilized):** 0.1 mL x 20 vials **SLP Diluent:** 5.0 mL x 2 vials **Standard (digested PG from *S. aureus*):** 0.5 mL x 1 vial
030-09903	Curdlan	1 g

CONSUMABLES

CODE	PRODUCT	SIZE	QUANTITY
294-35011	Bio Clean Tip Wako® Extend S II	200 µl	100 pcs
291-35021	Bio Clean Tip Wako® 200 II	200 µl	100 pcs
298-35031	Bio Clean Tip Wako® 1000 II	1,000 µl	100 pcs
293-35221	Bio Clean Plate Wako™	96 well	50 plates
292-32751	Limulus Test Tube-S with Aluminum Cap	Ø 12 x 75 mm	10 pcs x 8
293-26551	Limulus Test Tube-S	Ø 12 x 75 mm	10 pcs x 10
293-28251	Aluminum Caps-S	Ø 15 x 18 mm	10 pcs x 10

TESTING PROCEDURE

SLP-HS SINGLE REAGENT SET II
(IN COMBINATION WITH TOXINOMETER® ET-7000)



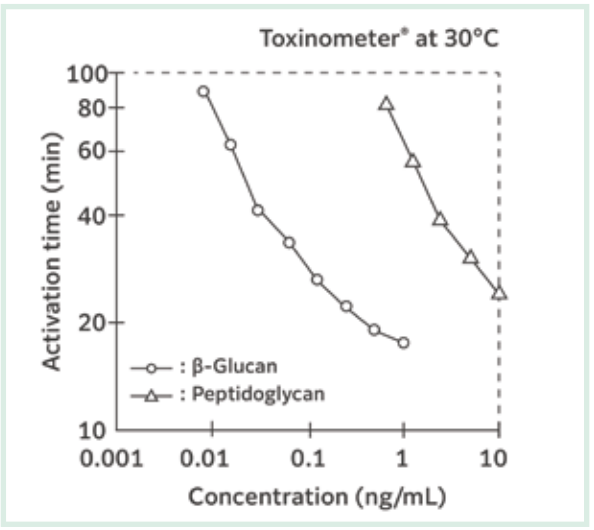


Fig. 3: Measuring example using Toxinometer®

Fig. 4: SLP-HS Single Reagent Set II

FIELDS OF APPLICATION

1. Study of the structure-activity relationship, biosynthesis, metabolism and etiological significance of PG
2. Analysis of water pollution
3. Microbial contamination testing of dialysate
4. Detection of fungal compounds in pharmaceuticals and medical devices, biologics and genetically-engineered products
5. Elucidation of the biological defense mechanism of insects

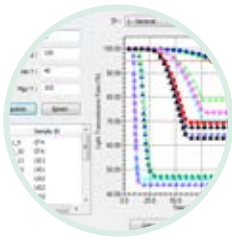
INSTRUMENT FEATURES

SAMPLE PREPARATION

+ No pretreatment required
+ Easy reagent handling

KINETIC COLORIMETRIC ASSAY

+ 16 sample positions, up to 128 with extension modules
+ Measurement starts automatically after sample is inserted



ANALYSIS OF REACTION TIME COURSE

+ Correlation with calibration curve
+ Absolute quantification of PG and BDG

+ Versatile application
+ Minimal maintenance necessary
+ FDA Part 11 compliant

Toxinometer® ET-7000:
190 × 420 × 130 mm (W x D x H), 6.3 kg

TEST FEATURES

CHARACTERISTICS

+ SLP (Silkworm Larvae Plasma reagent) based test principle
+ Specimen: any sample
+ Measurement time: 120 minutes at 30 °C

PERFORMANCE DATA

+ Assay Kinetic Colorimetric
+ PG isolated from *S. aureus* used as standard
+ Detection limit: 10 pg/mL (PG), 1 pg/mL (BDG)
+ Analysis automated through Toximaster® software
+ Indication: FDA Title 21 Part 11 compliant

For further information on our products
or to place an order, please contact us.

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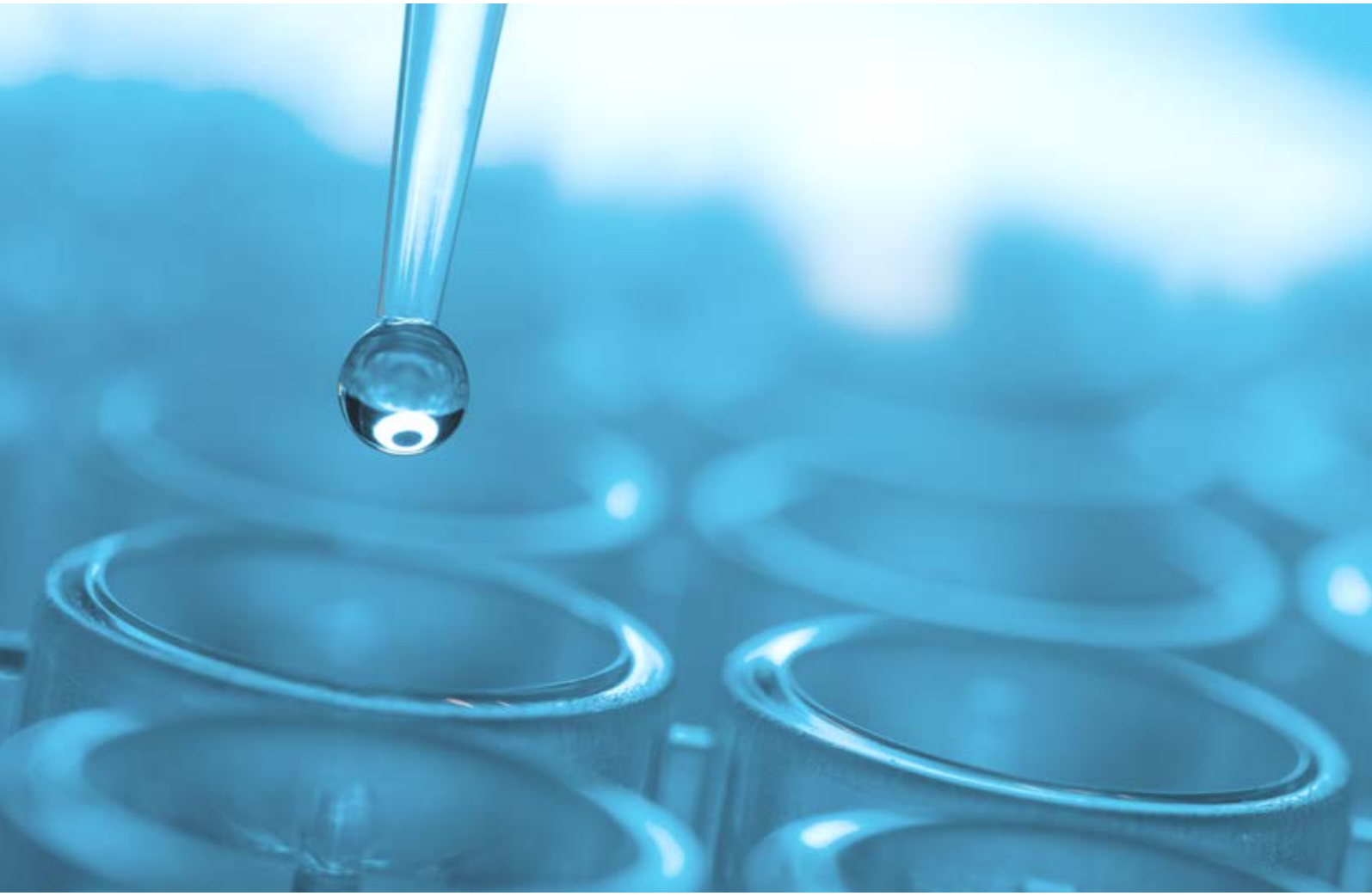

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