

SPECTROZYME® LAL

REF 200/086

Description

A chromogenic substrate for assaying the presence of gram-negative bacterial endotoxins.

Formula: $\text{CH}_3\text{O}-\text{CO}-\text{D}-\text{CHA}-\text{Gly}-\text{Arg}-\text{pNA}\cdot\text{AcOH}$
 $(\text{C}_{25}\text{H}_{38}\text{N}_8\text{O}_7 \cdot \text{C}_2\text{H}_4\text{O}_2)$

Molecular Weight: 622.7

Chemical Name: Methoxycarbonyl-D-cyclohexylalanyl-glycyl-arginine-para-nitroanilide acetate

Composition: Enzymatically digestible substrate
 colyophilized with glycine

Extinction Coefficient: $\epsilon_{405\text{ nm}} = 9650 \text{ M}^{-1}\cdot\text{cm}^{-1}$

Purity, via RP-HPLC: $\geq 95\%$

Solubility: Up to 25 mg/mL in water

Assay Conditions/Substrate Kinetics

Enzyme activity is determined by measuring the increase in absorbance of the free chromophore (pNA) generated per unit time at a wavelength of 405 nm. At excess substrate concentrations, the rate at which the absorbance increases due to the amount of chromophore released is linearly related to enzyme concentration. Measurement can be made either through acid quenching of the reaction (end-point method), or through use of a kinetic recording spectrophotometer (initial-rate-method).

Using the following conditions, levels of bacterial endotoxins may be measured:

Buffer: 0.05 M Tris-HCl, 0.2 M NaCl, 0.1% PEG 8000, pH 8.8

Temperature: 37°C

Substrate: 0.8 mM (in buffer) REF 200/086

Enzyme: Endotoxin Standard, 0 EU/mL – 1.0 EU/mL

Assay: (Read OD of solutions at a wavelength of 405 nm.)

1. Add 50 µL of LAL Reagent + 50 µL Standard or Sample.
2. Incubate for 20 minutes at 37°C.
3. Add 100 µL of SPECTROZYME LAL.
4. Incubate for 3 minutes at 37°C.
5. Add 100 µL of acetic acid to stop the reaction.

Presentation

REF 200/086 amber glass vial containing 100 µmoles (62.27 mg) of lyophilized, digestible substrate.

Reconstitution

Reconstitute with pyrogen free water only to create a stock solution with a concentration of 2.0 mM. Dilute further to the working concentration with a buffer of 0.05 M Tris-HCl, 0.2 M NaCl, 0.1% PEG 8000, pH 8.8. The typical working concentration for SPECTROZYME® LAL is 0.8 -1.25 mM.

Storage and Stability

Lyophilized substrate may be stored in the dark at 2° - 8°C up to the expiration date stated on the vial. Protect from moisture by allowing to reach room temperature prior to opening vial.

Reconstituted substrate may be stored for 1 week at room temperature, 2 months at 2° - 8°C, or for up to 6 months frozen at -20°C (Aliquot and freeze. Do not submit to freeze-thaw cycles).

Warnings and Precautions

For Research Use Only.

CONT	N-Methoxycarbonyl-D-cyclohexylalanyl-glycyl-L-arginine-4-nitroanilide acetate
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Warning

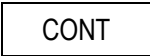
Hazard Statements:	H315 Causes skin irritation.
	H319 Causes serious eye irritation
	H335 May cause respiratory irritation
Precautionary Statements:	P261 Avoid breathing dust.
	P264 Wash thoroughly after handling.
	P280 Wear protective gloves/eye protection
	P337 + P313 If eye irritation persists: Get medical advice/attention
	P403 + P233 Store in a well-ventilated place. Keep container tightly closed.

References

1. Bussey, D. M. and Tsuji, K. *Journal of Parenteral Science and Technology* 1984, **38**: 228-233.
2. Lindsay, G. K., et al. *Journal of Clinical Microbiology* 1989, **27**: 947-951.
3. Itsuji, K. and Martin, P A. *Pharmaceutical Manufacturing* 1984, 34-38.
4. Schadewald, L. K., et al. *Journal of Parenteral Science and Manufacturing* 1990, 44: 50-53.

LAL= Limulus Amebocyte Lysate

Definition of Symbols

	Consult instructions for use
	Manufacturer
	Refer to Safety Data Sheet
	Contains...
	Temperature Limitation: Store at 2°C to 8°C
	Lot Number
	Expiration Date
	Catalog Number