FT-MS6690

HRP labeled GS-II Lectin

Product Description

Name: Pure Griffonia simplicifolia lectin (GS-II), Horseradish

Peroxidase conjugated

Catalog Number: FP-MS6690 1 mg purified GS-I HRP / 1 ml Buffer

Carbohydrate Specificity: Terminal α- or β-N-Acetylglucosamine. The specific linkage of the

N-Acetylglucosamine to the subterminal carbohydrate plays an important

role in lectin binding.

Inhibitory Carbohydrate: N-Acetylglucosamine

Activity: 5-10 μ g/ml will agglutinate T_k polyagglutinable cells.

Buffer: 0.01M Phosphate - 0.15M NaCl containing 0.5 mM CaCl2, pH 7.2 - 7.4

Storage: Store liquid material frozen in aliquots in amber vials or covered with foil.

Avoid freeze thaw cycles. Clarify by centrifugation. Protect from light and moisture. No preservatives have been added. Sodiumazide will inactivate the enzyme, peroxidase.

Stability: The liquid material is stable for at least 1 year when stored frozen in aliquots.

Directions for use

Remarks

Calcium is REQUIRED for binding.

0.5mM Calcium is the maximum concentration in Buffer that will not form a white precipitate.

Procedure

The following is a general Procedure and Trouble-Shooting Guide. The information is provided only for your convenience.

Chemical Principle

Peroxidase + $H_2O_2 \rightarrow Complex$

Complex + AH_2 (donor) \rightarrow Peroxidase + H_2O + A (colored)

Assays Reagents

BUFFER: 0.01M Sodium phosphate, pH 6.0.

ENZYME: Dilute with Buffer. Acceptable dilution: 1-2 μg/ml.

DYE: 1% o-dianisidine in methanol prepared fresh daily. Store in amber bottle or wrapped in foil.

SUBSTRATE: Prepare 0.3% H_20_2 solution in deionized or distilled water from stock H_20_2 solution Prior to use dilute to a final concentration 0.003% in Buffer.

Procedure

- 1. Add 0.05 ml of DYE to 6.0 ml of SUBSTRATE. Add 2.9 ml to Reaction test tube and 2.9 ml to Control test tube
- 2. At time=0, add 100μl of diluted ENZYME to Reaction tube and 100μl PBS to Control tube. Mix thoroughly.
- 3. Measure and record optical density at 460nm (OD460) every 15 seconds for 3 minutes, or take the end point reading after 3 minutes by stopping the reaction with 100µl of concentrated NaN₃.
- 4. Use this value to determine the rate of change in absorbance per minute.



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Enzyme Activity Calculations

One unit of peroxidase activity is that amount of enzyme decomposing 1 μ mole of peroxide/minute at 25°C. 11.3 x 10³ cm⁻¹ is the molar absorbance of H₂O₂.

$$OD460/min = \frac{OD460/3\min - ODControl/3\min \text{unites}}{3\min \text{nutes}}$$

$$mg \ enzyme/ml \ reaction \ mixture = \frac{[enzyme\ dilution]}{30}$$

$$units/mg = \frac{OD460/min}{11.3\ x\ mg\ enzyme/ml\ reaction\ mixture}$$

Caution

Due to inhibitory sugar present in the conjugates solution, to dilute the Conjugate 50-100 times with buffer before assay.

References

- Murphy L. et al., J. Biol.Chem. 252:4739-4742 (1977)
- Shanker Iyer PN. et al, Arch. Biochem. Biophys., 177:330-333 (1976)

Related / associated products and documents

See BioSciences Innovations catalogue and e-search tool.

- Lectin List (or search conjugated lectins):
- ConA-Biotin, <u>FP-MS9690</u>; -FITC, <u>FP-47496A</u>;
 -Cy3, <u>FP-WT8680</u>,
- GS-I-FITC; FP-MS9020

- PNA-FITC, FP-BV4181
- WGA-biotin, <u>FP-MS5730</u>; -SR101, <u>FP-MS9540</u>; -FITC, <u>FP-CE8070</u>

Ordering information

<u>Catalog size quantities and prices may be found at www.interchim.com/</u> Please inquire for higher quantities (availability, shipment conditions).

For any information, please ask: FluoProbes® / Interchim; Hotline: +33(0)4 70 03 73 06

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