DATA SHEET

Alkaline Phosphatase Labeled Lectins

Catalog Number: **LA-7201-1**

Description: **Crude Cancer antennarius lectin (CCA) from California crab, Alkaline Phosphatase conjugated.**

Protein Concentration: 1 mg purified biotin / 1 ml Buffer. Reconstitute with Buffer to a concentration of 1mg/1ml.

Carbohydrate Specificity: 9-O-acetyl sialic acid and 4-O-acetyl sialic acid.

Activity: Horse and rabbit erythrocytes will not react with human cells.

Buffer: 0.05MTris-0.1MNaCl-0.01MCaCl2, pH 7.2 containing 0.05%BSA.

Chemical Used for Conjugation: Alkaline Phosphatase.

Storage: Store liquid refrigerated at 5-8ºC in aliquots. DO NOT FREEZE! (20-50% Glycerol has been added to prevent freezing)

Stability: The liquid material is stable for at least 1 year when stored refrigerated in aliquots with 0.05% sodium azide added as a preservative.

Caution: The aluminum seals have sharp edges and the vial itself may have cracks which can cause lacerations. Use caution when opening the vial.

References: 1.Ravindranath,M.H. et al. (1985). J. Biol. Chem. 260:8850-8856.

2.Ravindranath,M.H. et al. (1987) Meth. Enzy. 138:520-527

3.Ravindranath M.H.. et al. J.Biol Chem 263:2079-2086/

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Procedure for use:

Chemical Principle:

$$\text{Orthophosphoric Monoester} + H_2O \xrightarrow{\text{En}} \text{Alcohol} + H_3PO_4$$

Assay Reagents:

BUFFER: 0.1 M Tris buffer, pH 8.2.

ENZYME: Dilute with 0.1 M Tris Buffer.
Acceptable dilution: 5-20 µg/ml.

SUBSTRATE: 0.001 M p-nitrophenylphosphate (P-NPP).

Procedure:

1. Add 2.9 ml substrate 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 Tris to Control tube. Mix thoroughly.
3. Measure and record optical density at 410 nm OD(410) every 15 seconds for 3 minutes, or take end point reading after 3 minutes by stopping reaction with 100µl of 5.0 M NaOH.
4. Use the OD(410) measurement to determine the rate of change in absorbance per minute.

Enzyme Activity Calculations:

One unit of activity is the amount of enzyme to decompose 1 µmole of P-NPP/minute at 25°C. 1.62×10^4 cm^{-1} is the molar absorbance of P-NPP.

$$\text{OD(410) / min} = \frac{\text{OD(410) / 3min - OD(410) Control / 3 minutes}}{\text{3 minutes}}$$

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

$$\text{units / mg} = \frac{\text{OD(410) / min}}{1.62 \times 10^4 \text{ ml reaction mixture}}$$

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