



### 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: 0.05M Tris-0.1M NaCl-0.01M CaCl<sub>2</sub>, pH 7.2 containing 0.05% BSA.

Chemical Used for Conjugation: Alkaline Phosphatase.

Storage: Store liquid refrigerated at 5-8°C 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.

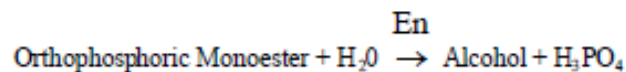
References: 1. Ravindranath, M.H. et al. (1985). J. Biol. Chem. 260:8850-8856.

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

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

Procedure for use:

**Chemical Principle:**



**Assay Reagents:**

BUFFER: 0.1 M Tris buffer, pH 8.2.

ENZYME: Dilute with 0.1 M Tris Buffer.

Acceptable dilution: 5-20  $\mu\text{g/ml}$ .

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 100 $\mu\text{l}$  of diluted ENZYME to Reaction tube and 100 $\mu\text{l}$  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 100 $\mu\text{l}$  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  $\mu\text{mole}$  of P-NPP/minute at 25°C.  $1.62 \times 10^4 \text{ cm}^{-1}$  is the molar absorbance of P-NPP.

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

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

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

GMP