

## Product Information

Version 4.5, Revision 2016-11-21

### A1130 - Alkaline Phosphatase, 2-Component System

Product:	<b>Alkaline Phosphatase, 2-Component System</b>
Catalog Number:	A1130
Concentration:	30 units/ $\mu$ l solution in 50% Glycerol, containing 5mM Tris, 5mM magnesium chloride and 0.1 mM zinc chloride, pH approximately 7.0.
Storage:	Store at 4°C. <b>DO NOT FREEZE</b>
Description:	This system contains Alkaline Phosphatase at <b>30 units/<math>\mu</math>l</b> concentration along with a second tube, containing a dilution/storage buffer. This concentration is suitable for conjugation to secondary antibodies or cellular components such as biotin or avidin. For DNA dephosphorylation, diluted concentration of 3 units/ $\mu$ l is recommended. For most applications, 0.5 $\mu$ l of diluted enzyme will dephosphorylate up to 20 pmol of 5' ends in a 50 $\mu$ l reaction volume at 37°C. The enzyme at 3 units/ $\mu$ l is stable for up to one year when stored at 4°C. Do not Freeze.
Dilution/Storage Buffer:	50% Glycerol, 5 mM Tris, 5 mM MgCl <sub>2</sub> , 0.1 mM ZnCl <sub>2</sub> , pH 7.0

## Protocols

### I. Dephosphorylation of DNA 5' termini<sup>1,2</sup>

#### Reagents:

- Alkaline Phosphatase
- Dilution/Storage Buffer

#### Required reagents not included:

- Dephosphorylation Reaction Buffer
  - 20 mM Tris-Cl, pH 8.0
  - 1 mM MgCl<sub>2</sub>
  - 1 mM ZnCl<sub>2</sub>
- 1-20 pmol 5' termini from linearized DNA vector

#### 1. Dilute alkaline phosphatase:

0.5  $\mu$ l of alkaline phosphatase at a concentration of 3 U/ $\mu$ l is sufficient to dephosphorylate about 20 pmol of 5' ends in a reaction volume of 50  $\mu$ l. Specific activity varies by lot and is listed on the product label and on the accompanying certificate of analysis.

Example: If activity is 30,000 units/ml it should be diluted 1:10 to achieve a working concentration of 3Units/ $\mu$ l. Prepare sufficient volume to have a final concentration of 1.5 units activity for 20 pmol 5'-termini.

#### 2. Incubate at 37°C for 60 minutes

#### 3. DNA recovery: Dephosphorylated DNA may be recovered by standard procedures including phenol/ chloroform extraction or by gel purification.

**Note:** Inactivation/inhibition of Alkaline Phosphatase activity

Heat inactivation: Heat to 75° C for 10 minutes in the presence of 5 mM EDTA to inactivate 95% of activity.

Inhibitors: Chelating agents, arsenate, cysteine, iodine, inorganic phosphate, pyrophosphate, diisopropyl phosphate, triphenylphosphate, diisopropyl fluorophosphate and L-phenylalanine.

## II. Conjugation of Alkaline Phosphatase to Antibodies<sup>3,4</sup>

### Reagents:

- Alkaline Phosphatase
- Dilution/Storage Buffer

### Required reagents not included:

- Antibody
- Sodium phosphate, pH 6.8
- 1% Glutaraldehyde
- 1 M Ethanolamine, pH 7.0
- PBS

1. Combine 10 mg antibody with 5 mg of alkaline phosphatase in a total volume of 1 ml.
2. Dialyze against 0.1 M sodium phosphate, pH 6.8 for 12 hours to remove free amines.
3. In a fume hood, add 0.05 ml of 1% glutaraldehyde and stir gently for 5 minutes.
4. Incubate for 3 hours at room temperature.
5. Add 0.1 ml of 1 M ethanolamine, pH 7.0.
6. Incubate for 2 hours at room temperature.
7. Dialyze against PBS for 12 hours at 4°C with 3 changes of PBS.
8. Centrifuge for 20 minutes at 40,000G to remove precipitates
9. Decant supernatant and store at 4°C in 50% glycerol, 1 mM ZnCl<sub>2</sub>, 1 mM MgCl<sub>2</sub> and 0.02% sodium azide.

### References:

<sup>1</sup>Moessner, E. et al., Z. Physiol.Chhem. 361, 543

<sup>2</sup>Sambrook, J. et al., Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory (1989), p 5.72, 6.22-6.47 and E.3-E13.

<sup>3</sup>O'Sullivan, M.J., and Marks, V., Meth.Enzymol., 73, 147-166 (1981)

<sup>4</sup>Harlow, E., and Lane, D., Antibodies: A Laboratory Manual, Cold Spring harbor Laboratory Press (Cold Spring Harbor, NY: 1988) Chapter 9, p.349.

**\* For laboratory research purpose and/or in vitro use only, and it is not to be used in humans.**