



cell signaling solutions

## Certificate of Analysis

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### Histone H1

(substrate for kinase assays: 95% pure)

Catalog # 14-155

Lot # 23770

**Product Description:** Purified as a lysine rich fraction from calf thymus. Purity  $\geq$  95% as judged by SDS PAGE and coomassie blue staining 3 $\mu$ g of protein.

**Sterility:** Sterilized through a 0.2 $\mu$ -membrane filter and packaged aseptically.

**Formulation:** 20mg of Histone H1 in 1ml sterile water. Frozen solution.

**Storage and Stability:** Stable for 1 year at -20°C from date of shipment. Aliquot to avoid repeated freezing and thawing.

**FOR RESEARCH USE ONLY  
NOT FOR USE IN HUMANS**

### Quality Control Testing and Research Applications

**Kinase Assay:** Histone H1 is an effective substrate for a number of serine/threonine kinases. This lot was tested by using PKC theta, active (Catalog # 14-444) to phosphorylate 10 $\mu$ g histone H1. Test results are shown to the right. A previous lot was tested by using cdk2/cyclin A (Catalog # 14-407).

PKC theta, active	Histone H1	Mean CPM	Comments
None	10 $\mu$ g	4,956	Substrate background
5 $\mu$ g	None	156,752	PKC theta background
5 $\mu$ g	10 $\mu$ g	2,040,931	PKC theta dependent phosphorylation of Histone H1 + background

#### Application Reference:

1. Sambucetti, L., *et al.*, J. Biol. Chem. **274**: 34940-34947, 1999.

#### General Reference:

1. de Nooij, E.H. and Westenbrink, H.G.K. Biochim.Biophys.Acta **62**: 608, 1968.

### Kinase Assay Protocol

#### Stock Solutions:

1. Assay Dilution Buffer II (ADBII, Catalog # 20-111): 20mM MOPS, pH 7.2, 25mM  $\beta$ -glycerophosphate, 1mM sodium orthovanadate, 1mM dithiothreitol, 1mM  $\text{CaCl}_2$ .
2. PKC theta, active: Use 0.5-5.0 $\mu\text{g}$  per assay point. Just prior to conducting an assay, dilute the required amount of enzyme with dilution buffer to a final concentration of 0.05-0.5 $\mu\text{g}/\mu\text{l}$ . Slowly pipette up and down several times to ensure sufficient mixing.
3. Histone H1 Substrate (Catalog # 14-155): Use at a final concentration of 1mg/ml; dilute in ADBII.
4. PKC Lipid Activator (Catalog # 20-133): 0.5mg/ml phosphatidylserine and 0.05mg/ml diglycerides in ADBII.
5. [ $\gamma$ - $^{32}\text{P}$ ]ATP: Stock 1mCi/100 $\mu\text{l}$  (3000Ci/mmol, obtained from DuPont-NEN). Make 10 $\mu\text{l}$  aliquots (100 $\mu\text{Ci}/\text{vial}$ ). Before starting the assay, dilute an aliquot with 90 $\mu\text{l}$  of 75mM magnesium chloride and 500 $\mu\text{M}$  ATP in ADBII.

#### Assay Procedure:

1. Add 10 $\mu\text{l}$  of the Histone H1 substrate to a microcentrifuge tube.
2. Add 20 $\mu\text{l}$  of ADBII to the tube.
3. Add 10 $\mu\text{l}$  of the PKC lipid activator. **The lipid activator must be sonicated on ice for at least one minute before use.**
4. Add **0.5-5.0 $\mu\text{g}$  (10 $\mu\text{l}$ ) of diluted PKC theta** solution.
5. Add 10 $\mu\text{l}$  of diluted [ $\gamma$ - $^{32}\text{P}$ ]ATP mixture.
6. Incubate for 10 minutes at 30°C.
7. Transfer a 25 $\mu\text{l}$  aliquot to the center of a numbered P81 paper.
8. Wash the assay squares with 0.75% phosphoric acid.
9. Wash the assay squares once with acetone.
10. Transfer the assay squares to a scintillation vial and add scintillation cocktail.
11. Read in scintillation counter. Compare CPM of enzyme samples to CPM of control samples without substrate (background control).

**Technical Note:** Allow the radiolabeled substrate to bind to the filter paper for 30 seconds before immersing the paper into a 50ml conical tube containing 40ml 0.75% phosphoric acid. Gently shake the assay squares for 5 minutes on a rotator. Discard the wash in a liquid radioisotope waste container, (dispose of per institutional regulations) and repeat the wash step twice. Wash the squares in 20ml of acetone for 5 minutes. Drain and add scintillation cocktail.