



cell signaling solutions

Certificate of Analysis

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Histone H1

(substrate for kinase assays: 95% pure)

Catalog # 14-155

Lot # 25186

Product Description: Purified as a lysine rich fraction from calf thymus. Purity \geq 95% as judged by SDS PAGE and Coomassie blue staining 1 μ g of protein.

Sterility: Sterilized through a 0.2 μ -membrane filter and packaged aseptically.

Formulation: 20mg of Histone H1 in 1.25ml 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 μ g histone H1. Test results are shown to the right. Previous lots have been tested by using cdk2/cyclin A (Catalog # 14-407) and cdk2/cyclin A, active (Catalog # 14-448).

PKC theta, active	Histone H1	Mean CPM	Comments
None	10 μ g	14,821	Substrate background
5 μ g	None	227,166	PKC theta background
5 μ g	10 μ g	3,635,607	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 β -glycerophosphate, 1mM sodium orthovanadate, 1mM dithiothreitol, 1mM CaCl_2 .
2. PKC theta, active: Use 0.5-5.0 μg 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. [γ - ^{32}P]ATP: Stock 1mCi/100 μl (3000Ci/mmol, obtained from PerkinElmer, Cat. # BLU002A). Make 10 μl aliquots (100 $\mu\text{Ci}/\text{vial}$). Before starting the assay, dilute an aliquot to 1 $\mu\text{Ci}/\mu\text{l}$ with 90 μl of 75mM magnesium chloride and 500 μM ATP in ADBII.

Assay Procedure:

1. Add 10 μl of the **Histone H1** substrate to a microcentrifuge tube.
2. Add 20 μl of ADBII to the tube.
3. Add 10 μl of the PKC lipid activator. **The lipid activator must be sonicated on ice for at least one minute before use.**
4. Add **10 μl (0.5-5.0 μg) of diluted PKC theta** solution.
5. Add 10 μl of diluted [γ - ^{32}P]ATP mixture.
6. Incubate for 10 minutes at 30°C.
7. Transfer a 25 μl 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.