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Certificate of Analysis

Histone H1

(substrate for kinase assays: 95% pure)

Catalog # 14-155

Lot # 21706

Product Description: Purified as a lysine rich fraction¹. Purity = 95% as judged by SDS PAGE and silver staining 1µg of protein.

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

Formulation: 20mg of Histone H1 packaged in 4 vials; each vial contains 5mg 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 cdk2/cyclin A, active (Catalog # 14-407) to phosphorylate 3µg histone H1. Test results are shown to the right.

A previous lot was tested by using PKCα (Catalog # 14-232) to phosphorylate 10µg histone H1.

cdk2/cyclin A, active	Histone H1	Mean CPM	Comments
None	3µg	15,238	Background
20U	None	16,280	cdk2/cyclin A autophosphorylation + background
20U	3µg	1,864,875	cdk2, cyclin A dependent phosphorylation of Histone H1 + background

General References:

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 I (ADBI, Catalog # 20-108): 20mM MOPS, pH 7.2, 25mM β -glycerol-phosphate, 1mM sodium orthovanadate, 1mM dithiothreitol, 5mM EDTA.
2. cdk2/cyclin A active: Use 10-40 Units per assay.
OR
PKC α (Catalog # 14-232): 10-25ng per assay point. Add 41 μ l **Enzyme Dilution Buffer** (Catalog # 20-146) to the 5 μ g of PKC α . Slowly pipette up and down several times to ensure good mixing. Just prior to conducting an assay, dilute the required amount of enzyme 1:10 with additional Enzyme Dilution Buffer and, again, pipette up and down slowly several times to ensure good mixing. This gives a final concentration of **10ng/ml** of PKC α .
3. Histone H1 Substrate: Use at a final concentration of 1mg/ml per assay in ADBI.
4. PKC Lipid Activator (Catalog # 20-133): 0.5mg/ml phosphatidylserine and 0.05mg/ml diglycerides in ADBI.
5. [γ -³²P]ATP: Stock 1mCi/100 μ l (3000Ci/mmol, obtained from DuPont-NEN). Make 10 μ l aliquots (100 μ Ci/vial). Before starting the assay, dilute an aliquot with 90 μ l of 75mM magnesium chloride and 500 μ M ATP in ADBI.

Assay Protocol Summary:

1. Add **3ml of Histone H1** to a microcentrifuge tube.
2. Add 2 μ l of ADBI to a microcentrifuge tube.
3. a. Add 10 μ l of the lipid activator. (**The lipid activator must be sonicated on ice for at least a minute before use**) and then add 25ng (2.5 μ l) of purified, diluted PKC α enzyme per tube..
OR
b. Add 10 μ l (20 Units) of cdk2/cyclin A, active.
4. Add 10 μ l of diluted [γ -³²P]ATP mixture.
5. Incubate for 10 minutes at 30°C.
6. Transfer a 25 μ l aliquot to the center of a numbered P81 paper.
7. Wash the assay squares with 0.75% phosphoric acid.
8. Wash the assay squares once with acetone.
9. Transfer the assay squares to a scintillation vial and add scintillation cocktail.
10. Read in scintillation counter. Compare CPM of enzyme samples to CPM of control samples without substrate (background control).