

Histone H1

(substrate for kinase assays: 95% pure)

Catalog # 14-155

Lot # 18939

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

Formulation: 20mg of Histone H1 packaged in 4 vials; each vial contains 5mg Histone H1 in 1ml sterile water. Frozen solution.

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

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 PKCa (Catalog # 14-232) to phosphorylate 10µg histone H1.

PKCa Enzyme	Histone H1	Mean CPM	Comments
None	10µg	3,451	Background
25ng	None	3,603	PKCa autophosphorylation + background
25ng	10µg	495,800	PKCa 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 II (ADBII): 20mM MOPS, pH 7.2, 25mM β -glycerophosphate, 1mM sodium orthovanadate, 1mM dithiothreitol, 1mM CaCl_2 .
2. PKCa: 10-25ng per assay point. Add 41 μl **Enzyme Dilution Buffer** (Catalog # 20-146) to the 5 μg of PKCa. 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/ μl** of PKCa.
3. Histone H1 Substrate (Catalog # 14-155): Use at a final concentration of 1mg/ml per assay in ADBII.
4. Protein Kinase C Lipid Activator: 0.5mg/ml phosphatidylserine and 0.05mg/ml diglycerides in ADBII.
5. [γ - ^{32}P]ATP: Stock 1mCi/100 μl (3000Ci/mmol, obtained from DuPont-NEN). Make 10 μl aliquots (100 μCi /vial). Before starting the assay, dilute an aliquot with 90 μl of 75mM magnesium chloride and 500 μM ATP in ADBII.

Assay Protocol Summary:

1. Add 10 μl of **Histone H1 Substrate** to a microcentrifuge tube.
2. Add 20 μl of ADBII to a microcentrifuge tube.
3. Add 10 μl of the lipid activator. **The lipid activator must be sonicated on ice for at least a minute before use.**
4. Add 25ng (2.5 μl) of purified, diluted PKCa enzyme per tube.
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).