

Certificate of Analysis

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Histone H1

(substrate for kinase assays)

Catalog # 14-155

Lot # DAM1467560

Product Description: Purified as a lysine rich fraction from calf thymus. Purity >90% as judged by SDS PAGE and Coomassie blue staining 1µg of protein. MW = 32kDa.

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

Formulation: 20mg of Histone H1 in 1ml sterile, distilled 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 # 14-448) to phosphorylate 2.5µg histone H1. Test results are shown to the right. Previous lots have been tested by using PKC θ, active (Catalog # 14-444).

cdk2/cyclin A, active	Histone H1	Mean CPM	Comments
None	2.5µg	1,349	Substrate background
100ng	None	1,596	cdk2/cyclin A background
100ng	2.5µg	49,244	cdk2/cyclin A dependent phosphorylation of Histone H1 + background

Application Reference:

1. Sambucetti, L., *et al.*, J. Biol. Chem. **274**: 34940-34947, 1999.
2. Chellaiah, M. A., *et al.*, J. Biol. Chem. **278**: 29086-97, 2003.
3. Chung, H. K., *et al.*, J. Biol. Chem. **278**: 28079-88, 2003.
4. Negishi, Y., *et al.*, J. Biol. Chem. **276**: 33249-56, 2001.

General Reference:

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

Kinase Assay Procedure

Stock Solutions:

1. 5X Assay Dilution Buffer (ADB): 40mM MOPS pH7.0, 1mM EDTA.
2. cdk2/cyclin A, active: Dilute to 40ng/ μ l with 20mM MOPS pH7.5, 1mM EDTA, 0.03% Brij-35, 5% glycerol, 1mg/ml BSA, 0.1% β -mercaptoethanol. Use 2.5 μ l per assay point.
3. Histone H1: Prepare a 0.5mg/ml stock solution using 1X ADB. Use 5 μ l per assay point for a final concentration of 0.1mg/ml per assay point.
4. [γ -³²P]ATP: Stock 1mCi/100 μ l (3000Ci/mmol, obtained from PerkinElmer, Cat. # BLU002A). Before starting the assay, dilute an aliquot to 1 μ Ci/ μ l with 90 μ l of 75mM MgCl₂ and 500 μ M cold ATP in 20mM MOPS, pH 7.2, 25mM β -glycerol phosphate, 5mM EGTA, 1mM sodium orthovanadate, 1mM dithiothreitol

Assay Protocol:

1. Add 5 μ l of 5X ADB per assay.
2. Add 5 μ l of the Histone H1 stock solution (0.1mg/ml final concentration).
3. Add **2.5 μ l of cdk2/cyclin A, active** (100ng).
4. Add 2.5 μ l of sterile distilled water.
5. Add 10 μ l of the diluted [γ -³²P]ATP.
6. Incubate for 10 minutes at 30°C with constant agitation.
7. Spot 20 μ l on the center of a 2cm x 2cm P81 paper square.
8. Wash assay squares three times with 0.75% phosphoric acid.
9. Wash assay squares once with acetone for 5 minutes.
10. Transfer assay squares to a scintillation vial and add 5ml scintillation cocktail.
11. Read in scintillation counter. Compare CPM of enzyme samples to CPM of control samples that contain substrate with no enzyme and enzyme with no substrate (background controls).

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.

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