

Histone H1
(Bovine calf thymus)

Catalog # 14-155

Lot # 16294

Product Description: Histones are purified from bovine calf thymus using the procedure of Panyim, *et al.*^{2,3} In this procedure, calf thymus is homogenized, centrifuged, rehomogenized, washed 5-7 times, and the resulting pellet extracted with 0.25M HCl. The supernatant is precipitated with cold acetone and histone H1 is purified from the whole histone preparation by the procedure of Oliver, *et al.*¹ that employs treatment with 70% perchloric acid, 0.2M H₂SO₄, and acetone precipitation.

Physical Form: Frozen solution.

Quantity and Formulation: 20mg of Histone H1 contained in 4 vials; each vial contains 5mg Histone H1 in 1.0ml sterile water.

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

Reference:

1. Oliver, D., *et al.*, Biochem. J. **129**: 349-353, 1972
2. Panyim, S., *et al.*, J. Biol. Chem. **246**: 4206-4215, 1971a
3. Panyim, S., *et al.*, J. Biol. Chem. **246** 7557-7560, 1971b

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Quality Control Testing and Research Applications

Kinase Assays: Histone H1 is an effective substrate for a number of serine/threonine kinases. This lot was tested by using cdc2 kinase (Catalog # 14-203) to phosphorylate histone H1.

Kinase Assay Protocol

Stock Solutions:

1. Assay Dilution Buffer (ADB): 20mM MOPS, pH7.2; 25mM β -glycerophosphate; 5mM EGTA, 1mM sodium orthovanadate; 1mM dithiothreitol.
2. Magnesium/ATP Cocktail: 500 μ M cold ATP and 75 mM magnesium chloride in ADB.
3. [γ -³²P]ATP: Stock 1mCi/100 μ l (3000Ci/mole, 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 Magnesium/ATP Cocktail.
4. cdc2 kinase: 25-100ng per assay tube.
5. cdc2 Substrate: Dilute Histone H1 with ADB to a working concentration of 1mg/ml.

Assay Procedure:

1. Add 10 μ l of ADB per assay.
2. Add 10 μ l of cdc2 kinase (25-100 ng/assay).
3. Add 10 μ l of Histone H1 (10 μ g/40 μ l assay).
4. Add 10 μ l of [γ -³²P]ATP mixture.
5. Incubate for 10 minutes at room temperature.
6. Blot 25 μ l aliquot on 2cm x 2cm P81 paper.
7. Wash assay squares three times with 0.75% phosphoric acid.
8. Wash assay squares once with acetone. Allow assay squares to dry.
9. Transfer assay squares to vial and add 5ml scintillation cocktail.
10. Count in scintillation counter. Compare CPM of enzyme samples to CPM of control samples that contain no enzyme (background control).

CDC2 KINASE	HISTONE H1	MEAN CPM	COMMENTS
None	10 μ g	3,578	Background
25ng	None	3,329	cdc2 autophosphorylation
25ng	10 μ g	139,286	cdc2 dependent phosphorylation of Histone H1