

Product Datasheet

HeLa Cell Nuclear Extract (orb348671)

Description

HeLa Cell Nuclear Extract

Conjugation

Unconjugated

Tested

SDS-PAGE, WB

Applications

Preservatives

Preservative: None. Stabilizer: 10% (v/v) Glycerol. 1X SDS-PAGE Sample Buffer (62.5 mM Tris HCl, 2% SDS, 10% Glycerol and 0.005% bromophenol blue, pH 6.8)

Form/Appearance

Liquid (sterile filtered)

Concentration

1.0 mg/ml

Storage

Store HeLa Cell Nuclear Extract at -70° C or COLDER. For extended storage, aliquot Nuclear Extract to minimize freeze/thaw cycles.

Note

For research use only

Application notes

ready-to-use nuclear extracts are especially prepared as positive controls for separation by SDS-PAGE and subsequent western blot analysis. Nuclear extracts are supplied in denaturing buffer without dissociating agents. Heat nuclear extract to 95°C for 5 minutes and rapidly cool. If dissociating conditions are desired add reducing agent prior to heating. The recommended loading volume per lane is 10-30l depending on the size format of your gel.

Purity

The cells were grown in Dulbecco's medium supplemented with 10% fetal bovine serum. Cells were washed with PBS and then incubated on ice in modified RIPA buffer, containing 150 mM sodium chloride, 50 mM Tris HCl, pH 7.4, 1 mM EDTA, 1.0% NP-40, 0.5% sodium deoxycholic acid , 0.1% SDS and 0.01% (w/v) sodium azide to lyse the cells. Protein integrity was ensured using a cocktail of protease inhibitors with broad specificity for the inhibition of aspartic, cysteine, and serine proteases as well as aminopeptidases (0.1 mM AEBSF HCl, 0.08 µM Aprotinin, 5 µM Bestatin, 1.5 µM E-64, 2 µM Leupeptin Hemisulfate, 1 µM Pepstatin A). Phosphatase inhibitors 1 mM NaF and 1 mM Na3VO4 were also added. Cell debris was removed by centrifugation. Protein concentration was determined by a modified Lowry assay using a commercially

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