
Product Datasheet

HeLa Whole Cell Lysate Nocodazole Stimulated (orb348683)

Description

HeLa Whole Cell Lysate Nocodazole Stimulated

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 vial at -70° C or COLDER. For extended storage, aliquot contents to minimize freeze/thaw cycles.

Note

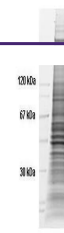
For research use only

Application notes

ready-to-use lysates are especially prepared as positive controls for separation by SDS-PAGE and subsequent western blot analysis. Lysates are prepared in denaturing buffer WITHOUT dissociating agents (i.e. no 2-mercaptoethanol or dithiothreitol has been added). Heat lysate 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 10-20 µl depending on the size format of your gel.

Purity

The cells were grown in DMEM supplemented with 10% FBS (Fetal Bovine Serum). Cells were treated with 3 µg/ml nocodazole for 1 h. Cells were washed in PBS and incubated on ice in modified RIPA buffer containing 150 mM sodium chloride, 50 mM Tris Cl, pH 7.4, 1 mM EDTA, 1.0% NP-40, 0.5% sodium deoxycholic acid and 0.1% SDS to lyse the cells. Protein integrity is 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 and 1 µM Pepstatin A). Phosphatase inhibitors sodium fluoride, sodium orthovanadate, sodium pyrophosphate and D-glycerophosphate were also added. Cell debris was removed by centrifugation. Protein concentration was determined by Lowry assay using a commercially available kit. The protein concentration was adjusted to 2 mg/ml and then an equal volume of 2X SDS-PAGE sample



Coomassie stained SDS-PAGE of 35 µg of ...