

Product Datasheet

A-172 Cell Nuclear Extract (orb348709)

Description

A-172 Cell Nuclear Extract

Conjugation

Unconjugated

Tested

ChIP, IP, WB

Applications

Preservatives

Preservative: None. Stabilizer: None. 1X RIPA Buffer with HALT Protease and Phosphatase Inhibitors

Form/Appearance

Liquid (sterile filtered)

Concentration

4.0 mg/ml

Storage

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

Note

For research use only

Application notes

Multi-purpose A-172 nuclear extracts are especially prepared as positive control for multiple assays including western blot, immunoprecipitation (IP), capture ELISA or other assays requiring native protein sample. For separation by SDS-PAGE and subsequent western blot analysis, lysates should be diluted by user to desired concentration in SDS-PAGE buffer with 2-mercaptoethanol or dithiothreitol as the reducing agent and heated to 95° C for 5 minutes. Sample is ready for use in immunoprecipitation and ELISA experiments, conditions should be optimized by the user. Rockland recommends its TrueBlot IP reagents for immunoprecipitation experiments.

Purity

A-172 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 Benztatin A). Phosphatase inhibitors 1 mM

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adjusted to 4 mg/ml in RIPA buffer containing protease and phosphatase