

Product Datasheet

HCT-116 Whole Cell Lysate (orb348712)

Description

HCT-116 Whole Cell Lysate

Conjugation

Unconjugated

Tested

ChIP, IP, SDS-PAGE, 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 lysate to minimize freeze/thaw cycles.

Note

For research use only

Application notes

Multi-purpose HCT-116 Whole Cell Lysate 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

HCT-116 cells were grown in McCoy'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 with 1X RIPA buffer.