

Human U-251 Whole Cell Lysate

Cat. No. U-251-017HCL **Lot. No.** (See product label)

SPECIFICATION

Product Overview

Ready-to-use whole cell lysates are derived from cell lines or tissues using highly refined extraction protocols to ensure exceptionally high quality, protein integrity and lot-to-lot reproducibility. All extracts are tested by SDS-PAGE using 4-20% gradient gels and immunoblot analysis using antibodies to key cell signaling components to confirm the presence of both high molecular weight and low molecular weight proteins.

Species

Human

Form

Liquid (sterile filtered)

Specificity

U-251 cells were grown in Minimum Essential Medium supplemented with 10% fetal bovine serum. Cells were washed with PBS and then incubated on ice in modified RIPA buffer 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 μ M Aprotinin, 5 μ M Bestatin, 1.5 μ M E-64, 2 μ M Leupeptin Hemisulfate, 1 μ M 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 BCA assay using a commercially available kit. Protein concentration was adjusted to 1 mg/mL using 2× SDS-PAGE sample buffer.

Applications

WB, SDS-PAGE

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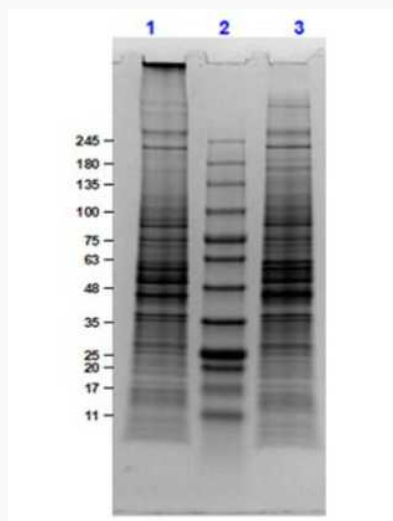
Cell Line	Human - Astrocytoma
Lysate Fractionation	Whole Cell Lysate
Lysate Stimulation	Not Stimulated
Culture Type	Tissue Culture
Induction	None (Control)
Concentration	1.0 mg/mL by BCA assay
Buffer	1× SDS-PAGE Sample Buffer (62.5 mM Tris HCl, 2% SDS, 10% Glycerol and 0.005% bromophenol blue, pH 6.8)
Preservative	None
Stabilizer	10% (v/v) Glycerol
Shipping	Dry Ice
Storage	Store vial at -70 centigrade or COLDER. For extended storage, aliquot contents to minimize freeze/thaw cycles.
Expiration	Expiration date is three months from date of receipt.

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SDS-PAGE



SDS-PAGE Results of U-251 Whole Cell Lysate.

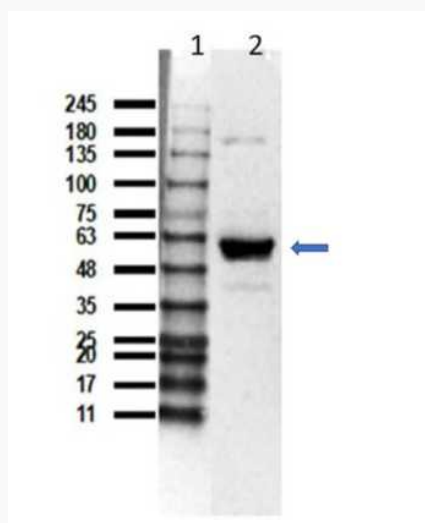
Lane 1: U-251 Whole Cell Lysate (10 µg) Reduced.

Lane 2: Molecular Weight Marker.

Lane 3: U 251 Whole Cell Lysate (10 µg) Non-Reduced.

Results show wide range of molecular weight bands with no sign of degradation.

Western Blot



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Western Blot results of Rabbit Anti-Beclin 1 Antibody using U-251 lysate.

Lane 1: Opal Prestain Molecular Weight Marker.

Lane 2: U251-MG whole cell lysate (35 µg) [+].

Primary Antibody: Anti-Beclin1 at 1:1000 overnight at 2-8 centigrade.

Secondary Antibody: Goat Anti-Rabbit IgG HRP at 1:40,000 for 60 min at RT.

Block: 5% BLOTTO/TBST.

Expected MW: ~51 kDa. Observed MW: ~53 kDa.

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