

Chinese Hamster K1 Whole Cell Lysate

Cat. No. K1-002CCL **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

Chinese Hamster

Form

Liquid

Specificity

The cells were grown in Ham's F12 medium containing 2 mM L-Gln and 1.5 g/L sodium bicarbonate supplemented with 10% FBS (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 Lowry assay using a commercially available kit. Protein concentration was adjusted to 2 mg/mL and then an equal volume of 2× SDS-PAGE sample buffer was added.

Applications

WB, SDS-PAGE

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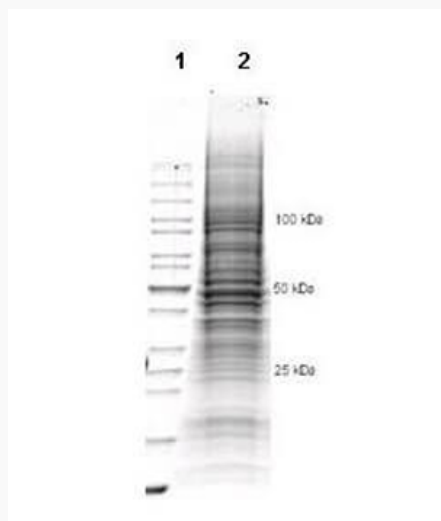
Cell Line	Chinese Hamster Ovary (CHO)
Lysate Fractionation	Whole Cell Lysate
Lysate Stimulation	Not Stimulated
Culture Type	Tissue Culture
Induction	None (Control)
Concentration	1.0 mg/mL by BCA assay
Buffer	1× SDS-PAGE Sample Buffer (62.5 mM Tris HCl, 2% SDS, 10% Glycerol and 0.005% bromophenol blue, 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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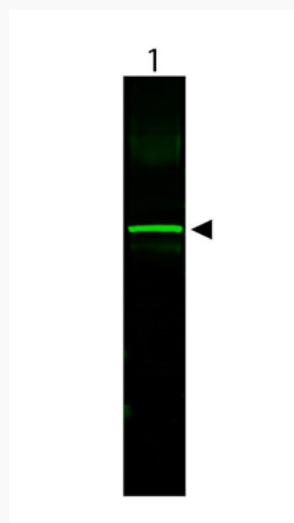
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SDS-PAGE



Coomassie stained SDS-PAGE of 35 μ L of CHO Whole Cell Lysate (Ready-to-Use) separated in a 4-20% gradient gel under reducing conditions (lane 2). Molecular weight standards are shown in lane 1.

WB



Western Blot showing detection of alpha tubulin in lane 1. CHO/K1 Whole Cell

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Lysate (10 µg) was run on a 4-20% gel, then transferred to 0.45 µm nitrocellulose. After blocking with 1% BSA-TTBS 30 min at 20 centigrade, primary antibody was used at 1:1000 overnight at 4 centigrade. Anti-Rabbit IgG (H&L) (GOAT) antibody IRDye800CW secondary antibody was used at 1:20,000 in Blocking Buffer for Fluorescent Western Blotting and imaged on the LiCor Odyssey imaging system. Arrow indicates correct 50 kDa molecular weight position expected for alpha tubulin.

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