Human Foreskin Fibroblast Whole Cell Lysate

Cat. No. FF-01HL **Lot. No.** (See product label)

SPECIFICATION

Product Overview	Human Foreskin Fibroblast Whole Cell Lysate
Species	Human
Source	Human Foreskin Fibroblast
Lysate preparation	The cells were grown in DMEM supplemented with 10% FBS (Fetal Bovine Serum). The lysate was prepared by first washing the cells in PBS. Washed cells were 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 and 0.1% SDS 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 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 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.
Recommended Usage	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).

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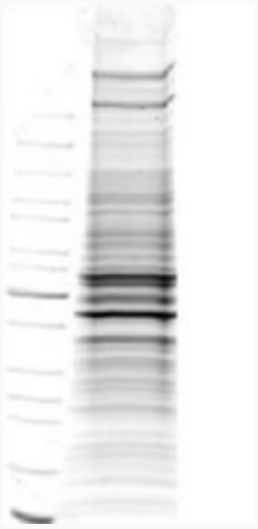
Storage	Store at 2-8 centigrade for continuous use. For extended storage, freeze working aliquots at -70 centigrade. Repeated freezing and thawing is not recommended. Under proper storage conditions the shelf life is half a year from the date of receipt.
Storage Buffer	1X SDS-PAGE Sample Buffer (62.5 mM Tris HCl, 2% SDS, 10% Glycerol and 0.005% bromophenol blue, pH 6.8)
Concentration	2 mg/mL
SDS-PAGE	

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