Item Name Hydrogen Peroxide Stimulated MCF-7 Whole Cell Lysate

Cat# HCL-2014

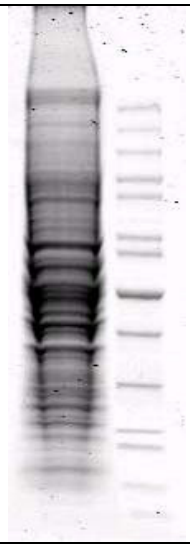
Size: 100 ug

Tissue type Epithelial (Mammary)

Source Human

Buffer: 1X SDS-PAGE Sample Buffer (62.5 mM Tris HCl, 2% SDS, 10% Glycerol and 0.005% bromophenol blue, pH 6.8)

Store Lysate is supplied at a concentration of 2 mg/ml. Store at 2-8˚C for continuous use. For extended storage, freeze working aliquots at -70˚C. Repeated freezing and thawing is not recommended. Under proper storage conditions the shelf life is half a year from the date of receipt.



Lysate preparation

The cells were grown in DMEM supplemented with 10% FBS (Fetal Bovine Serum). Cells were treated with 300 µM H_2O_2 at 37℃ for 15 minutes. The lysate was prepared by first washing the cells in PBS supplemented with EDTA and a cocktail of protease inhibitors (see below). Washed cells are 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, 0.25% sodium deoxycholic acid to lyse the cells. Protein integrity is ensured using a cocktail of protease inhibitors with broad specificity for the inhibition of aspartic, cysteine, and serine proteases as well as aminopeptidases (0.5 mM AEBSF HCl, 0.4 mM Aprotinin, 25 µM Bestatin, 7.5 µM E-64, 10 µM Leupeptin Hemisulfate and 5 µM Pepstatin A). In addition, 5mM sodium fluoride and 40mM B-glycerophosphate were added to inhibit phosphatase activity. Cell debris was removed by 0.45uM membrane filtration. Protein concentration was determined by Lowry assay using a commercially available kit. The protein concentration was adjusted to 2.0 mg/ml and then an equal volume of 2X SDSPAGE sample buffer was added.

All items are for in vitro research use only.

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).

Related items

#90100	Western blot recycling kit
80200	Western blot Kit for Rabbit Primary Antibodies, Chemilum. Substrate
80201	Western blot Kit for Goat Primary Antibodies, Chemilum. Substrate
80202	Western blot Kit for Mouse Primary Antibodies, Chemilum. Substrate
80215	NuGlo ECL Substrate Kit for Western blot
80400-100	Bovine Serum Albumin (BSA, protease-free) for western (makes ~2-L buffer @5% BSA)
80400-100	Bovine Serum Albumin (BSA, protease-free) for western (makes ~2-L buffer @5% BSA)
#ACTB12-M	Mouse monoclonal anti-beta actin IgG
#G3PDH12-M	Mouse monoclonal anti-G3PDH (GAPDH)

RIPA buffer, mol wt markers, Mouse, rat and human tissue lysates and pre-made protein blots are also available.

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Alpha Diagnostic Intl Inc., 6203 Woodlake Center Dr, San Antonio, TX 78244, USA; **Email**: service@4adi.com
☎ (800) 786-5777; (210) 561-9515; **Fax**: (210) 561-9544; **Web Site**: www.4adi.com