


Item Name MDA-MD-435S Whole Cell Lysate

Cat#	HCL-2017
Size:	100 ug
Tissue type	mammary gland ductal carcinoma
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.

	<p>Lysate preparation</p> <p>The cells were grown in Leibovitz's L-15 medium with 0.01 mg/ml insulin, 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 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 a modified Lowry assay using a commercially available kit. Protein concentration was adjusted to 2 mg/ml and then an equal volume of 2X SDS-PAGE sample buffer was added.</p> <p>All items are for in vitro research use only.</p>
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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.

HCL-2017 80711S

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