Item Name A431Cell Nuclear Extract

Cat# HCL-2015

Size: 100 ug

Tissue type A431 (epidermoid 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.



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 lysis buffer containing 10 mM HEPES, 60 mM KCl, 1.0 mM EDTA, 0.075% (v/v) NP40 and 1.0 mM DTT, pH 7.6. 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 (0.1 mM AEBSF HCl, 0.08 µM Aprotinin, 5 µM Bestatin, 1.5 µM E-64, 2 µM Leupeptin Hemisulfate and 1 µM Pepstatin A). Nuclei were then collected and washed in lysis buffer minus detergent. Nuclei were lysed by vortexing in extraction buffer containing 20 mM Tris-Cl, 1.5 mM $MgCl_2$, 0.42 M NaCl, 0.2 mM EDTA, and 25% (v/v) glycerol, pH 8.0, supplemented with protease inhibitors (see above). The lysate was clarified by centrifugation. 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 SDS-PAGE 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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