



Item Name Mouse 3T3 (NIH) Nuclear Extract

Cat# MCL-2000

Size: 100 ug

Tissue type NIH Swiss Webster Mouse embryo

Source Mouse

Buffer: 1X SDS-PAGE Sample Buffer (0.05 mM Tris HCl, 2% SDS, 10% Glycerol and 0.1% 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.

Recommended Usage

Western blot-Load 5-20 ug/lane for testing with various antibodies. Heat at 95oC for 2-5 minutes prior to loading on gels.

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 MgCl₂, 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.

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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