HeLa Cell Nuclear Extract - TNFa Stimulated - W09-001-A86

Code: W09-001-A86 **Size:** 200 µg

Product Description: HeLa Cell Nuclear Extract - TNFa Stimulated - W09-001-A86

PhysicalState: Liquid (sterile filtered)

Label	Unconjugated
Buffer	1X SDS-PAGE Sample Buffer (62.5 mM Tris HCl, 2% SDS, 10% Glycerol and 0.005% bromophenol blue, pH 6.8)
Stabilizer	10% (v/v) Glycerol
Preservative	None
Storage Condition	Store HeLa Cell Nuclear Extract TNF alpha Stimulated at -70° C or COLDER. For extended storage, aliquot Nuclear Extract to minimize freeze/thaw cycles.
Synonyms	HeLa Cell Nuclear Extract TNFa Stimulated, HeLa Nuclear Lysate TNF alpha Stimulated, HeLa TNF alpha Stimulated Nuclear Lysate, HeLa Lysate Tumor Necrosis Factor Nuclear Extract
Application Note	Ready-to-use nuclear extracts are especially prepared as positive controls for separation by SDS-PAGE and subsequent western blot analysis. Nuclear extracts are supplied in denaturing buffer without dissociating agents. Heat nuclear extract to 95° C for 5 minutes and rapidly cool. If dissociating conditions are desired add reducing agent prior to heating. The recommended loading volume per lane is 10-30 l depending on the size format of your gel.
Background	Ready-to-use nuclear extracts produced by Rockland Immunochemicals are derived from cell lines or tissues using highly refined extraction protocols to ensure exceptionally high quality, protein integrity and lot-to-lot reproducibility. All extracts are tested by SDS-PAGE using 4-20% gradient gels and immunoblot analysis using antibodies to key cell signaling components to confirm the presence of both high molecular weight and low molecular weight proteins.
Purity And Specificity	The cells were grown in DMEM supplemented with 10% FBS (Fetal Bovine Serum). Cells were treated with 0.2 µg/ml TNF for 30 min. 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.
Western Blot	User Optimized
Expiration	Expiration date is three (3) months from date of opening.

Related Products

200-301-268	Anti-AKT pS473 (MOUSE) Monoclonal Antibody - 200-301-268
610-4302	Anti-MOUSE IgG (H&L) (RABBIT) Antibody Peroxidase Conjugated - 610-4302
611-1302	Anti-RABBIT IgG (H&L) (GOAT) Antibody Peroxidase Conjugated - 611-1302
B304	NORMAL GOAT SERUM (NGS) - B304

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