Product Datasheet

A431 Cell Nuclear Extract (orb348679)

Description	A431 Cell Nuclear Extract
Conjugation	Unconjugated
Tested Applications	WB
Preservatives	None. 1X SDS-PAGE Sample Buffer (62.5 mM Tris HCl, 2% SDS, 10% Glycerol and 0.005% bromophenol blue, pH 6.8)
Form/Appearance	Liquid (sterile filtered)
Concentration	1.0 mg/ml
Storage	Store vial at -70° C or COLDER. For extended storage, aliquot contents to minimize freeze/thaw cycles.
Note	For research use only
Application notes	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.
Purity	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

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Source	Human