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Product Datasheet

HEK293 Whole Cell Lysate (orb348669)

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Description ^{nts.}	HEK293 Whole Cell Lysate	·····, ···, ···, ···, ···, ···, ···, ·
Conjugation	Unconjugated	
Tested Applications	SDS-PAGE, WB	
Preservatives	Preservative: None. Stabilizer: 10% (v/v) Glycerol. 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 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). Heat lysate 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 10-20 µl depending on the size format of your gel.	
Purity	HEK293 cells were grown in Dulbecco's medium 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 , 0.1% SDS and 0.01% (w/v) sodium azide 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.	

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