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Datasheet for W09-001-GV8

HEK293 Whole Cell Lysate MG-132 Treated

Overview

Description:	HEK293 Whole Cell Lysate - MG-132 Stimulated - W09-001-GV8
Item No.:	W09-001-GV8
Size:	500 µg
Applications:	SDS-PAGE
Origin:	Human

Product Details

Background:	Ready-to-use whole cell lysates 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.
Synonyms:	Hek-293 Lysate MG-132 treated, Cell Lysate, MG132 Stimulated Lysate, HEK293
Species of Origin:	Human

Target Details

Purity/Specificity:	HEK293 cells were grown in Dulbecco's medium supplemented with 10% fetal bovine serum. Cells were treated O/N with 0.2µg/mL of MG-132 in order to reduce degradation of ubiquitin-conjugated proteins by the proteasome. Cells were washed with PBS and then incubated on ice in modified RIPA buffer 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. Protein concentration was determined by a modified Lowry assay using a commercially available kit. Protein concentration was adjusted to 2 mg/ml and then an equal volume of 2X SDS-PAGE sample buffer was added.

Application Details

Tested Applications:	SDS-PAGE
Application Note:	W09-001-GV8 has been tested by SDS-PAGE. 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.
Assay Dilutions:	All assays should be optimized by the user. Recommended dilutions (if any) may be listed below.
WB:	User Optimized

Cell Line Data

Cell Line:	Human Epithelial (Kidney)
Lysate Fractionation:	Whole Cell Lysate
Lysate Stimulation:	MG-132
Culture Type:	Tissue Culture
Induction:	MG-132

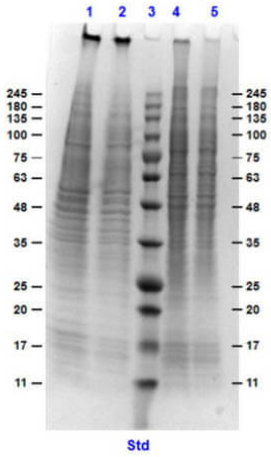
Formulation

Physical State:	Liquid
Concentration:	1.0 mg/mL by modified Lowry assay
Buffer:	1X SDS-PAGE Sample Buffer (62.5 mM Tris HCl, 2% SDS, 10% Glycerol and 0.005% bromophenol blue, pH 6.8)
Preservative:	None
Stabilizer:	10% (v/v) Glycerol

Shipping & Handling

Shipping Condition:	Dry Ice
Storage Condition:	Store vial at -70° C or COLDER. For extended storage, aliquot contents to minimize freeze/thaw cycles.
Expiration:	Expiration date is three (3) months from date of receipt.

Images



SDS-PAGE
SDS-PAGE of HEK293 Whole Cell Lysate MG-132 Stimulated. Lane 1: HEK293 unstimulated reduced. Lane 2: HEK293 MG-132 stimulated reduced. Lane 3: Molecular Weight Marker MB-210-0500. Lane 4: HEK293 unstimulated non-reduced. Lane 5: HEK293 MG-132 stimulated non-reduced. Load 10ng.

Disclaimer

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