Product Datasheet

ERK2 Knockout A549 Cell Lysate
NBP3-18632

Unit Size: 100 ug

Store at -70C. Avoid freeze-thaw cycles.

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ERK2 Knockout A549 Cell Lysate

Product Information	
Unit Size	100 ug
Concentration	mg/ml
Storage	Store at -70C. Avoid freeze-thaw cycles.
Preservative	No Preservative
Purity	Multi-step
Buffer	1X RIPA Buffer with HALT Protease and Phosphatase Inhibitors

Product Description	
Description	MAPK1 (ERK2) knockout A549 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 uM Aprotinin, 5 uM Bestatin, 1.5 uM E-64, 2 uM Leupeptin Hemisulfate, 1 uM 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 BCA using a commercially available kit. Protein concentration was adjusted to 2 mg/ml with modified 1X RIPA buffer. MAPK1 (ERK2) knockout A549 Clone 15 contains knockout deletions on all three copies of the MAPK1 (ERK2) gene in A549 cells. Each copy contains the same 104bp deletion induced by CRISPR/Cas9. The deletion occurs in exon 2 and disrupts the sequence between amino acids 59 to 94. These mutations induce a frame-shift and result in early stop codons. Validated by Sanger sequencing and Western blot. Clone 15 Lysate Fractionation: Whole Cell Lysate Lysate Stimulation: Not Stimulated Culture Type: Tissue Culture Induction: None (Control)
Gene ID	5594
Gene Symbol	MAPK1
Species	Human

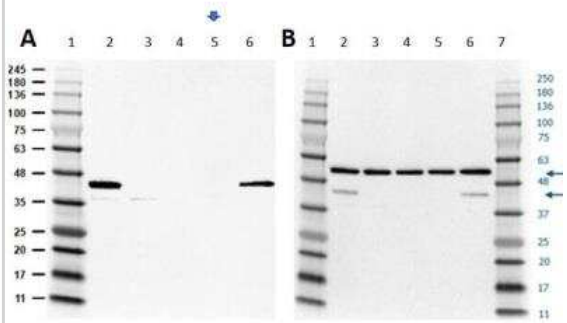
Preparation Method	MAPK1 (ERK2) knockout A549 cells were grown in Dulbecco's medium supplemented with 10% fetal bovine serum. Cells were washed with PBS and then incubated on ice in modified RIPA buffer, containing 150 mM sodium chloride, 50 mM Tris HCl, pH 7.4, 1 mM EDTA, 1.0% NP-40, 0.5% sodium deoxycholic 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 uM Aprotinin, 5 uM Bestatin, 1.5 uM E-64, 2 uM Leupeptin Hemisulfate, 1 uM 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 BCA using a commercially available kit. Protein concentration was adjusted to 2 mg/ml with modified 1X RIPA buffer. MAPK1 (ERK2) knockout A549 Clone 15 contains knockout deletions on all three copies of the MAPK1 (ERK2) gene in A549 cells. Each copy contains the same 104bp deletion induced by CRISPR/Cas9. The deletion occurs in exon 2 and disrupts the sequence between amino acids 59 to 94. These mutations induce a frame-shift and result in early stop codons. Validated by Sanger sequencing and Western blot.
Lysate Type	Knockout A549 Cell
Lysate Life Stage	Adult
Lysate Subcellular Fraction	Whole

Product Application Details

Applications	Western Blot, ELISA, Immunoprecipitation, SDS-Page, Chromatin Immunoprecipitation (ChIP)
Recommended Dilutions	Western Blot, ELISA, Immunoprecipitation, SDS-Page, Chromatin Immunoprecipitation (ChIP)
Application Notes	This product has been tested by SDS-PAGE and western blot and is suitable for use in Western blot, ELISA, Immunoprecipitation and ChIP. No detection of expected band at ~44kDa is observed in MAPK1 (ERK2) knockout A549 when compared with unmodified A549 cell lysates by Western blot.

Images

Western Blot: ERK2 Knockout A549 Cell Lysate [NBP3-18632] - Lane 1: Opal Prestained MW Marker Lane 2: A549 WCL Parental. Lane 3: A549 MAPK1 KO Clone 4. Lane 4: A549 MAPK1 KO Clone 10. Lane 5: A549 MAPK1 KO Clone 15. Lane 6: HeLa WCL Parental Load: 10ug lysate/lane. Primary Antibody [Blot A] Anti-MAPK1(ERK2) ~44kDa; [Blot B] stripped and re-probed with Anti-Tubulin ~50kDa; at 1ug/mL overnight at 2-8C. Secondary Antibody: Goat Anti-Rabbit IgG HRP at 1:30,000 for 1hr at RT. Block: BlockOut Buffer for 1hr at RT. No detection of expected band at ~44kDa is observed in MAPK1 (ERK2) knockout A549 when compared with unmodified A549 cell lysates by Western blot.



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Products Related to NBP3-18632

NBP1-30309	Recombinant Human ERK2 His Protein
210-TA-005	TNF-alpha [Unconjugated]
NBP1-47842	ERK2 Antibody (OTI6E5)
D6050	IL-6 [HRP]

Limitations
This product is for research use only and is not approved for use in humans or in clinical diagnosis.
Lysates are guaranteed for 6 months from date of receipt.

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