#11843 Store at -20°C

Cell Fractionation Antibody Sampler Kit

☑ 1 Kit
(4 x 40 µl)

Cell Signaling TECHNOLOGY®

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New 09/12

For Research Use Only. Not For Use In Diagnostic Procedures.

Products Included	Product #	Quantity	Mol. Wt.	Isotype
AIF (D39D2) XP® Rabbit mAb	5318	40 µl	67 kDa	Rabbit IgG
Histone H3 (D1H2) XP® Rabbit mAb	4499	40 µl	17 kDa	Rabbit IgG
MEK1/2 (D1A5) Rabbit mAb	8727	40 µl	46 kDa	Rabbit IgG
Vimentin (D21H3) XP® Rabbit mAb	5741	40 µl	57 kDa	Rabbit IgG
Anti-rabbit IgG, HRP-linked Antibody	7074	100 µl		Goat

See www.cellsignal.com for individual component applications, species cross-reactivity, dilutions and additional application protocols.

Storage: Supplied in 10 mM sodium HEPES (pH 7.5), 150 mM NaCl, 100 µg/ml BSA, 50% glycerol and less than 0.02% sodium azide. Store at –20°C. *Do not aliquot the antibodies.*

Recommended Antibody Dilutions:
Western blotting 1:1000

Please visit www.cellsignal.com for a complete listing of recommended companion products.

Description: The Cell Fractionation Antibody Sampler Kit provides an economical means for determining the purity of each distinctly separated subcellular fraction by western blot using Cell Signaling Technology's Cell Fractionation Kit #9038. This antibody sampler kit includes enough primary antibody to perform at least four western blots per primary antibody.

Background: Knowledge of the subcellular location of a protein may reveal the potential role it plays in a variety of cellular processes. Antibodies in the Cell Fractionation Antibody Sampler Kit can be used as a marker to ensure that each subcellular fraction is efficiently separated from the next. MEK1 and MEK2, also called MAPK or Erk Kinases, are dual-specificity protein kinases that function in a mitogen activated protein kinase cascade (1). Apoptosis-inducing factor (AIF) is a ubiquitously expressed flavoprotein that plays a critical role in caspase-independent apoptosis (2). Core histone protein, H3 is one of the primary building blocks of chromatin that makes up the nucleosome (3). Vimentin is a cell-specific intermediate filament with mesenchyme origin that contributes to making up of the cytoskeleton (4). MEK1/2, AIF, histone H3, and vimentin localize to the cytoplasm, mitochondria, nucleus, and cytoskeleton, respectively.

Specificity/Sensitivity: Each antibody in the Cell Fractionation Antibody Sampler Kit recognizes endogenous levels of its respective target protein. The antibodies do not cross-react with other family members.

Each antibody has been validated using the **Cell Fractionation Kit #9038**. Expression of these proteins may vary in different cells and tissues.

Source/Purification: Rabbit monoclonal antibodies are produced by immunizing animals with a synthetic peptide corresponding to residues surrounding Ala520 of human AIF protein, the carboxy terminus of human histone H3 protein, residues surrounding Ala220 of human MEK1 protein, and residues surrounding Arg45 of human vimentin protein.

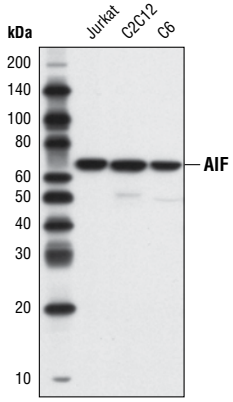
Background References:

(1) Crews, C.M. et al. (1992) *Science* 258, 478-80.

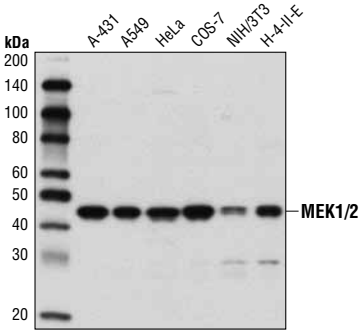
(2) Lipton, S.A. and Bossy-Wetzel, E. (2002) *Cell* 111, 147-50.

(3) Workman, J.L. and Kingston, R.E. (1998) *Annu Rev Biochem* 67, 545-79.

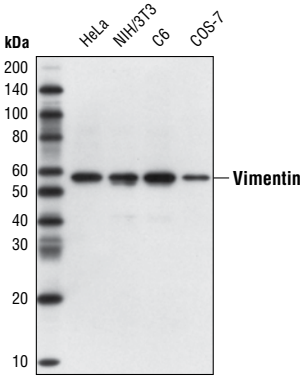
(4) Eng, L.F. et al. (2000) *Neurochem Res* 25, 1439-51.



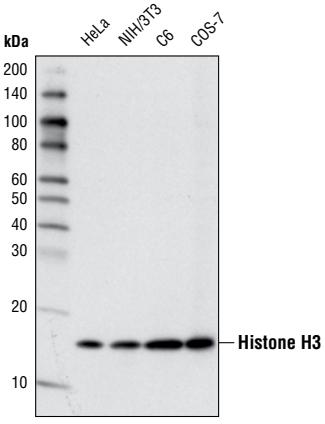
*Western blot analysis of extracts from Jurkat, C2C12, and C6 cells using **AIF (D39D2) XP® Rabbit mAb #5318**.*



*Western blot analysis of extracts from various cell lines using **MEK1/2 (D1A5) Rabbit mAb #8727**.*



*Western blot analysis of extracts from various cell lines using **Vimentin (D21H3) XP® Rabbit mAb #5741**.*



*Western blot analysis of extracts from various cell lines using **Histone H3 (D1H2) XP® Rabbit mAb #4499**.*

Applications Key: **W**—Western **IP**—Immunoprecipitation **IHC**—Immunohistochemistry **ChIP**—Chromatin Immunoprecipitation **IF**—Immunofluorescence **F**—Flow cytometry **E-P**—ELISA-Peptide

Species Cross-Reactivity Key: **H**—human **M**—mouse **R**—rat **Hm**—hamster **Mk**—monkey **Mi**—mink **C**—chicken **Dm**—D. melanogaster **X**—Xenopus **Z**—zebrafish **B**—bovine **Dg**—dog **Pg**—pig **Sc**—S. cerevisiae **All**—all species expected Species enclosed in parentheses are predicted to react based on 100% homology.

#11843

Western Immunoblotting Protocol (Primary Antibody Incubation in BSA)

For Western blots, incubate membrane with diluted antibody in 5% w/v BSA, 1X TBS, 0.1% Tween-20 at 4°C with gentle shaking, overnight.

A Solutions and Reagents

NOTE: Prepare solutions with Milli-Q or equivalently purified water.

1. 1X Phosphate Buffered Saline (PBS)
2. **1X SDS Sample Buffer:** 62.5 mM Tris-HCl (pH 6.8 at 25°C), 2% w/v SDS, 10% glycerol, 50 mM DTT, 0.01% w/v bromophenol blue or phenol red
3. **Transfer Buffer:** 25 mM Tris base, 0.2 M glycine, 20% methanol (pH 8.5)
4. **10X Tris Buffered Saline (TBS):** To prepare 1 liter of 10X TBS: 24.2 g Tris base, 80 g NaCl; adjust pH to 7.6 with HCl (use at 1X).
5. Nonfat Dry Milk (weight to volume [w/v])
6. **Blocking Buffer:** 1X TBS, 0.1% Tween-20 with 5% w/v nonfat dry milk; for 150 ml, add 15 ml 10X TBS to 135 ml water, mix. Add 7.5 g nonfat dry milk and mix well. While stirring, add 0.15 ml Tween-20 (100%).
7. **Wash Buffer:** 1X TBS, 0.1% Tween-20 (TBS/T)
8. Bovine Serum Albumin (BSA)
9. **Primary Antibody Dilution Buffer:** 1X TBS, 0.1% Tween-20 with 5% BSA; for 20 ml, add 2 ml 10X TBS to 18 ml water, mix. Add 1.0 g BSA and mix well. While stirring, add 20 µl Tween-20 (100%).
10. **Phototope®-HRP Western Blot Detection System** #7071**:** Includes biotinylated protein ladder, secondary anti-rabbit (#7074) antibody conjugated to horseradish peroxidase (HRP), anti-biotin antibody conjugated to HRP, LumiGLO® chemiluminescent reagent and peroxide.
11. Prestained Protein Marker, Broad Range (Premixed Format) #7720
12. Biotinylated Protein Ladder Detection Pack #7727
13. **Blotting Membrane:** This protocol has been optimized for nitrocellulose membranes, which CST recommends. PVDF membranes may also be used.

B Protein Blotting

A general protocol for sample preparation is described below.

1. Treat cells by adding fresh media containing regulator for desired time.
2. Aspirate media from cultures; wash cells with 1X PBS; aspirate.
3. Lyse cells by adding 1X SDS sample buffer (100 µl per well of 6-well plate or 500 µl per plate of 10 cm diameter plate). Immediately scrape the cells off the plate and transfer the extract to a microcentrifuge tube. Keep on ice.
4. Sonicate for 10–15 seconds to shear DNA and reduce sample viscosity.
5. Heat a 20 µl sample to 95–100°C for 5 minutes; cool on ice.
6. Microcentrifuge for 5 minutes.
7. Load 20 µl onto SDS-PAGE gel (10 cm x 10 cm).

NOTE: CST recommends loading prestained molecular weight markers (#7720, 10 µl/lane) to verify electrotransfer and biotinylated protein ladder (#7727, 10 µl/lane) to determine molecular weights.

8. Electrotransfer to nitrocellulose or PVDF membrane.

C Membrane Blocking and Antibody Incubations

NOTE: Volumes are for 10 cm x 10 cm (100 cm^2) of membrane; for different sized membranes, adjust volumes accordingly.

1. (Optional) After transfer, wash nitrocellulose membrane with 25 ml TBS for 5 minutes at room temperature.
2. Incubate membrane in 25 ml of blocking buffer for 1 hour at room temperature.
3. Wash three times for 5 minutes each with 15 ml of TBS/T.
4. Incubate membrane and primary antibody (at the appropriate dilution) in 10 ml primary antibody dilution buffer with gentle agitation overnight at 4°C.
5. Wash three times for 5 minutes each with 15 ml of TBS/T.
6. Incubate membrane with HRP-conjugated secondary antibody (1:2000) and HRP-conjugated anti-biotin antibody (1:1000) to detect biotinylated protein markers in 10 ml of blocking buffer with gentle agitation for 1 hour at room temperature.
7. Wash three times for 5 minutes each with 15 ml of TBS/T.

D Detection of Proteins

1. Incubate membrane with 10 ml LumiGLO® (0.5 ml 20X LumiGLO®, 0.5 ml 20X Peroxide and 9.0 ml Milli-Q water) with gentle agitation for 1 minute at room temperature.

NOTE: LumiGLO® substrate can be further diluted if signal response is too fast.

2. Drain membrane of excess developing solution (do not let dry), wrap in plastic wrap and expose to x-ray film. An initial 10-second exposure should indicate the proper exposure time.

NOTE: Due to the kinetics of the detection reaction, signal is most intense immediately following LumiGLO® incubation and declines over the following 2 hours.