#12693 Store at -20°C

Endodermal Lineage Marker Antibody Sampler Kit

☑ 1 Kit
(8 x 40 µl)

Cell Signaling TECHNOLOGY®

Orders ■ 877-616-CELL (2355)
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For Research Use Only. Not For Use In Diagnostic Procedures.

Products Included	Product #	Quantity	Mol. Wt.	Isotype
AFP (D12C1) Rabbit mAb	4448	40 µl	65 kDa	Rabbit IgG
N-Cadherin Antibody	4061	40 µl	140 kDa	Rabbit IgG
EOMES Antibody	4540	40 µl	70 kDa	Rabbit IgG
FoxA2/HNF3β (D56D6) XP® Rabbit mAb	8186	40 µl	50 kDa	Rabbit IgG
GATA-6 (D61E4) XP® Rabbit mAb	5851	40 µl	55 kDa	Rabbit IgG
HNF4α (C11F12) Rabbit mAb	3113	40 µl	52 kDa	Rabbit IgG
PDGF Receptor α (D13C6) XP® Rabbit mAb	5241	40 µl	190 kDa	Rabbit IgG
Sall4 (D16H12) Rabbit mAb	8459	40 µl	80, 142 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.

Description: The Endodermal Lineage Marker Antibody Sampler Kit provides an economical means of evaluating proteins expressed during endoderm development. This kit contains enough antibody to perform four western blot experiments per primary antibody.

Background: Two endodermal lineages develop during mammalian embryogenesis, the primitive endoderm of the blastocyst stage embryo and the definitive endoderm at gastrulation. The primitive endoderm gives rise to extra-embryonic lineages encompassing the visceral and the parietal endoderm. The definitive endoderm contributes to the respiratory and gastrointestinal tracts by forming the epithelial lining of the trachea, esophagus, lungs, stomach and intestines, and is a major component of many glands, including thyroid, thymus, pancreas and liver (1). Understanding molecular mechanisms that regulate early endodermal fates is seminal for the advance of stem cell research as they connect the transition from pluripotency to endoderm specification during mammalian development and contribute to the generation of clinically relevant cell types. FoxA2/HNF3β is a transcription factor essential for development of the endoderm and midline structures in mouse embryos (2,3). EOMES acts during gastrulation to promote the specification of the definitive endoderm (4). Markers of hepatic differentiation in the endoderm include expression of α-fetoprotein (AFP) and N-cadherin (5,6). HNF4α is involved in the differentiation of the visceral endoderm. GATA-6 lies upstream of HNF4 in a transcriptional cascade that regulates differentiation of the visceral endoderm and is also required for the establishment of the endodermally derived bronchial epithelium (7). Sall4 is required for the formation of the primitive endoderm from inner cell mass. It has been reported that extra-embryonic stem cell lines cannot be formed in Sall4-deficient blastocysts (8). PDGF receptor α is expressed in primitive endoderm derivatives throughout embryogenesis (9).

Specificity/Sensitivity: Each antibody recognizes endogenous total levels of its specific target protein. Sall4 (D16H12) Rabbit mAb recognizes endogenous levels of total Sall4A and Sall4B proteins.

Source/Purification: Monoclonal antibodies are produced by immunizing animals with a synthetic peptide corresponding to residues near the carboxy terminus of human AFP protein, residues surrounding Gly138 of human FoxA2/HNF3β protein, residues near the amino terminus of human GATA-6 protein, the sequence of human HNF4α protein, a recombinant protein corresponding to the PDGF receptor α extracellular domain, or residues surrounding Ala311 of human Sall4 protein. Polyclonal antibodies are produced by immunizing animals with a synthetic peptide corresponding to residues near the amino terminus of human N-cadherin protein or near the amino terminus of human EOMES protein. Polyclonal antibodies are purified by protein A and peptide affinity chromatography.

Background References:

(1) Wells, J.M. and Melton, D.A. (1999) *Annu Rev Cell Dev Biol* 15, 393-410.

(2) Weinstein, D.C. et al. (1994) *Cell* 78, 575-88.

(3) Ang, S.L. and Rossant, J. (1994) *Cell* 78, 561-74.

(4) Costello, I. et al. (2011) *Nat Cell Biol* 13, 1084-91.

(5) Zhao, D. et al. (2009) *PLoS One* 4, e6468.

(6) Meier, V. et al. (2006) *Comp Hepatol* 5, 2.

(7) Morrisey, E.E. et al. (1998) *Genes Dev* 12, 3579-90.

(8) Elling, U. et al. (2006) *Proc Natl Acad Sci USA* 103, 16319-24.

(9) Orr-Urtreger, A. et al. (1992) *Development* 115, 289-303.

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 antibody.*

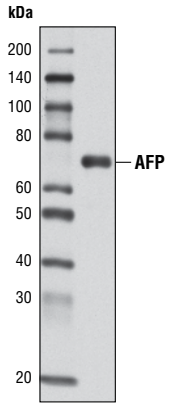
Recommended Antibody Dilutions:

Western blotting	1:1000

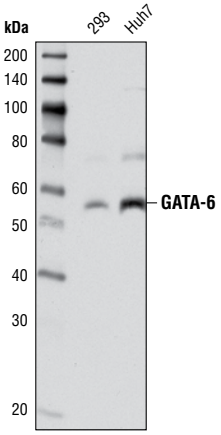
Please visit www.cellsignal.com for a complete listing of recommended companion products.

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

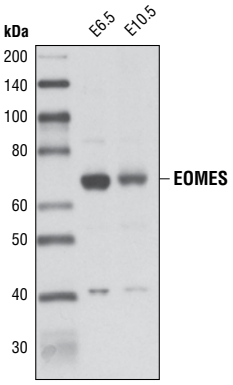
Species Cross-Reactivity Key: **H**—human **M**—mouse **R**—rat **Hm**—hamster **Mk**—monkey **Mi**—mink **C**—chicken **Dm**—D. melanogaster **X**—Xenopus **Z**—zebrafish **B**—bovine **Dg**—dog **Pg**—pig **Sc**—S. cerevisiae **All**—all species expected Species enclosed in parentheses are predicted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



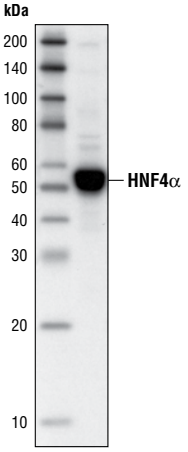
*Western blot analysis of extracts from Hep G2 cells using **AFP (D12C1) Rabbit mAb #4448**.*



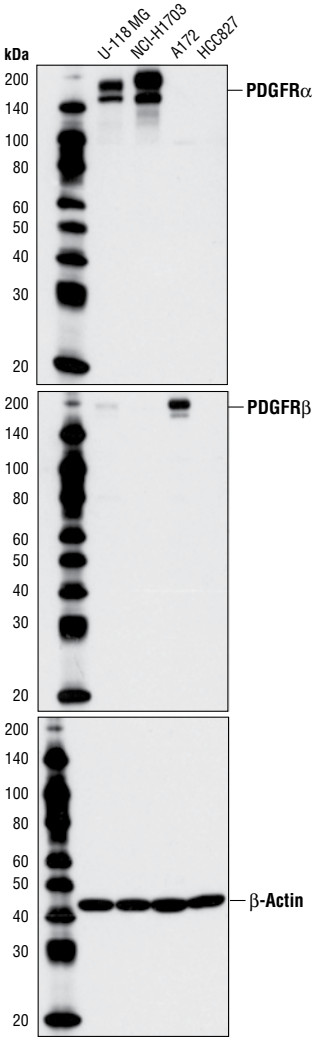
*Western blot analysis of extracts from Huh7 and 293 cells using **GATA-6 (D61E4) XP® Rabbit mAb #5851**.*



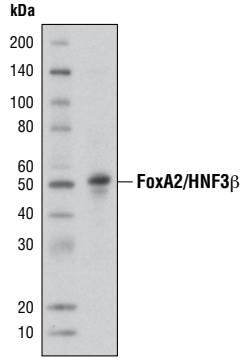
*Western blot analysis of extracts from E6.5 and E10.5 stage mouse embryos using **EOMES Antibody #4540.***



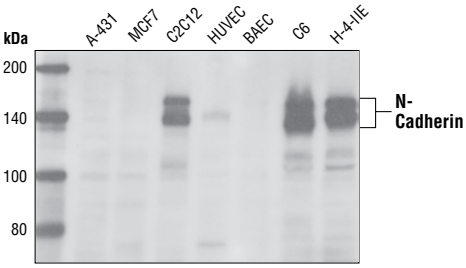
*Western blot analysis of extract from Hep G2 cells using **HNF4α (C11F12) Rabbit mAb #3113**.*



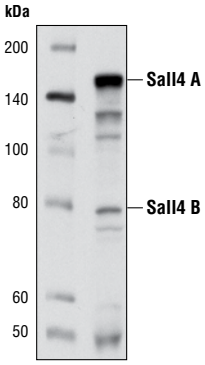
*Western blot analysis of extracts from various cell lines using **PDGF Receptor α (D13C6) XP® Rabbit mAb #5241** (upper), PDGF Receptor β (28E1) Rabbit mAb #3169 (middle), and β-Actin Antibody #4967 (lower).*



*Western blot analysis of extracts from Hep G2 cells using **FoxA2/HNF3β (D56D6) XP® Rabbit mAb #8186**.*



*Western blot analysis of extracts from various cell lines using **N-cadherin Antibody #4061**.*



*Western blot analysis of extracts from NCCIT cells using **Sall4 (D16H12) Rabbit mAb #8459**.*

#12693

Western Immunoblotting Protocol

For western blots, incubate membrane with diluted primary antibody in either 5% w/v BSA or nonfat dry milk, 1X TBS, 0.1% Tween® 20 at 4°C with gentle shaking, overnight.
NOTE: Please refer to primary antibody datasheet or product webpage for recommended primary antibody dilution buffer and recommended antibody dilution.

A. Solutions and Reagents

NOTE: Prepare solutions with reverse osmosis deionized (RODI) or equivalent grade water.

1. **20X Phosphate Buffered Saline (PBS):** (#9808) To prepare 1 L 1X PBS: add 50 ml 20X PBS to 950 ml dH_2O, mix.
2. **10X Tris Buffered Saline (TBS):** (#12498) To prepare 1 L 1X TBS: add 100 ml 10X to 900 ml dH_2O, mix.
3. **1X SDS Sample Buffer:** Blue Loading Pack (#7722) or Red Loading Pack (#7723) Prepare fresh 3X reducing loading buffer by adding 1/10 volume 30X DTT to 1 volume of 3X SDS loading buffer. Dilute to 1X with dH_2O.
4. **10X Tris-Glycine SDS Running Buffer:** (#4050) To prepare 1 L 1X running buffer: add 100 ml 10X running buffer to 900 ml dH_2O, mix.
5. **10X Tris-Glycine Transfer Buffer:** (#12539) To prepare 1 L 1X transfer buffer: add 100 ml 10X transfer buffer to 200 ml methanol + 700 ml dH_2O, mix.
6. **10X Tris Buffered Saline with Tween® 20 (TBST):** (#9997) To prepare 1 L 1X TBST: add 100 ml 10X TBST to 900 ml dH_2O, mix.
7. **Nonfat Dry Milk:** (#9999)
8. **Blocking Buffer:** 1X TBST with 5% w/v nonfat dry milk; for 150 ml, add 7.5 g nonfat dry milk to 150 ml 1X TBST and mix well.
9. **Wash Buffer:** (#9997) 1X TBST
10. **Bovine Serum Albumin (BSA):** (#9998)
11. **Primary Antibody Dilution Buffer:** 1X TBST with 5% BSA or 5% nonfat dry milk as indicated on primary antibody datasheet; for 20 ml, add 1.0 g BSA or nonfat dry milk to 20 ml 1X TBST and mix well.
12. **Biotinylated Protein Ladder Detection Pack:** (#7727)
13. **Prestained Protein Marker, Broad Range (Premixed Format):** (#7720)
14. **Blotting Membrane and Paper:** (#12369) This protocol has been optimized for nitrocellulose membranes. Pore size 0.2 µm is generally recommended.
15. **Secondary Antibody Conjugated to HRP:** anti-rabbit (#7074); anti-mouse (#7076)
16. **Detection Reagent:** LumiGLO® chemiluminescent reagent and peroxide (#7003) or SignalFire™ ECL Reagent (#6883)

B. Protein Blotting

A general protocol for sample preparation.

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 for a 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 sec to complete cell lysis and shear DNA (to reduce sample viscosity).
5. Heat a 20 µl sample to 95–100°C for 5 min; cool on ice.
6. Microcentrifuge for 5 min.
7. Load 20 µl onto SDS-PAGE gel (10 cm x 10 cm). **NOTE:** Loading of prestained molecular weight markers (#7720, 10 µl/lane) to verify electrotransfer and biotinylated protein ladder (#7727, 10 µl/lane) to determine molecular weights are recommended.
8. Electrotransfer to nitrocellulose membrane (#12369).

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.

I. Membrane Blocking

1. (Optional) After transfer, wash nitrocellulose membrane with 25 ml TBS for 5 min at room temperature.
2. Incubate membrane in 25 ml of blocking buffer for 1 hr at room temperature.
3. Wash three times for 5 min each with 15 ml of TBST.

II. Primary Antibody Incubation

1. Incubate membrane and primary antibody (at the appropriate dilution and diluent as recommended in the product datasheet) in 10 ml primary antibody dilution buffer with gentle agitation overnight at 4°C.
2. Wash three times for 5 min each with 15 ml of TBST.
3. Incubate membrane with the species appropriate HRP-conjugated secondary antibody (#7074 or #7076 at 1:2000) and anti-biotin, HRP-linked Antibody (#7075 at 1:1000–1:3000) to detect biotinylated protein markers in 10 ml of blocking buffer with gentle agitation for 1 hr at room temperature.
4. Wash three times for 5 min each with 15 ml of TBST.
5. Proceed with detection (Section D).

D. Detection of Proteins

1. Incubate membrane with 10 ml LumiGLO® (0.5 ml 20X LumiGLO® #7003, 0.5 ml 20X peroxide, and 9.0 ml purified water) or 10 ml SignalFire™ #6883 (5 ml Reagent A, 5 ml Reagent B) with gentle agitation for 1 min at room temperature.
2. Drain membrane of excess developing solution (do not let dry), wrap in plastic wrap and expose to x-ray film. An initial 10 sec exposure should indicate the proper exposure time. **NOTE:** Due to the kinetics of the detection reaction, signal is most intense immediately following incubation and declines over the following 2 hr.