#12854 Store at -20°C

SWI/SNF Complex Antibody Sampler Kit

☑ 1 Kit
(6 x 40 µl)

Cell Signaling TECHNOLOGY®

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New 08/13

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

Products Included	Product #	Quantity	Mol. Wt.	Isotype
ARID1A/BAF250A (D2A8U) Rabbit mAb	12354	40 µl	250 kDa	Rabbit IgG
Brg1 (A52) Antibody	3508	40 µl	220 kDa	Rabbit IgG
BRM (D9E8B) XP® Rabbit mAb	11966	40 µl	200 kDa	Rabbit IgG
SMARCC1/BAF155 (D7F8S) Rabbit mAb	11956	40 µl	155 kDa	Rabbit IgG
SMARCC2/BAF170 (D8O9V) Rabbit mAb	12760	40 µl	162, 170 kDa	Rabbit IgG
SNF5 (D9C2) Rabbit mAb	8745	40 µl	44 kDa	Rabbit IgG
Anti-rabbit IgG, HRP-linked Antibody	7074	100 µl		Goat

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.

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

Description: The SWI/SNF Complex Antibody Sampler Kit provides an economical means of detecting total protein from the SWI/SNF family members including ARID1A/BAF250A, Brg1, BRM, SMARCC1/BAF155, SMARCC2/BAF170 and SNF5. The kit contains enough primary antibody to perform four western blots per primary antibody.

Background: ATP-dependent chromatin remodeling complexes play an essential role in the regulation of various nuclear processes, such as gene expression, DNA replication, and repair (1,2). The SWI/SNF chromatin remodeling complex consists of more than 10 subunits with a single molecule of the ATPase catalytic subunit BRM or BRG1, but not both. The activities of these two subunits drive the disruption of histone-DNA contacts that lead to changes in accessibility of crucial regulatory elements within chromatin (2-5). The BRM/BRG1 containing SWI/SNF complexes are recruited to target promoters by transcription factors, such as nuclear receptors, p53, RB, and BRCA1 to regulate gene activation, cell growth, the cell cycle, and differentiation processes (1,6-9). BRM and BRG1 are also considered to be tumor suppressors and their expression levels are severely reduced in several cancer cell lines (10-13). SMARCC1/BAF155, SMARCC2/BAF170, and SNF5 are members of the core subunits of the SWI/SNF complex, which is necessary for efficient nucleosome remodeling by BRG1 *in vitro* (14). ARID1A/BAF250A is one of the accessory subunits of the SWI/SNF complex (15). SMARCC1, SNF5, and ARID1A are an essential part of the mouse embryonic stem cell specific SWI/SNF complex (esBAF). SMARCC1 is necessary for early embryogenesis, especially proper brain and visceral endoderm development (16-18). SNF5 is necessary for early embryogenesis and hepatocyte differentiation (19,20). ARID1A is critical for ES cell pluripotency and differentiation into mesoderm-derived cardiomyocytes and adipocytes (15). While SMARCC2 has been shown to be part of the SWI/SNF complex in non-pluripotent cells, it is absent in pluripotent embryonic stem (ES) cells. Expression of SMARCC2 has been shown to be up-regulated in neurons/neuronal progenitors upon differentiation of mouse ES cells with retinoic acid, and exogenous expression of SMARCC2 leads to loss of stem cell pluripotency and self renewal (21).

Specificity/Sensitivity: Each antibody in this kit recognizes endogenous levels of total protein for the specified target and does not cross-react with other family members. ARID1A/BAF250A (D2A8U) Rabbit mAb also cross-reacts with proteins of unknown origin at 65 kDa.

Source/Purification: Monoclonal antibody is produced by immunizing animals with a synthetic peptide corresponding to residues surrounding Gly1293 of human ARID1A/BAF250A protein, Gly264 of human BRM protein, Gly975 of human SMARCC1/BAF155 protein, Ile818 of human SMARCC2/BAF170 protein, or Gln244 of human SNF5 protein. Polyclonal antibodies are produced by immunizing animals with a synthetic peptide corresponding to residues surrounding Ala52 of human Brg1 protein. Polyclonal antibodies are purified by Protein A and peptide affinity chromatography.

Background References:

(1) Ho, L. and Crabtree, G.R. (2010) *Nature* 463, 474-84.
(2) Becker, P.B. and Hörz, W. (2002) *Annu Rev Biochem* 71, 247-73.
(3) Eberharter, A. and Becker, P.B. (2004) *J Cell Sci* 117, 3707-11.
(4) Bowman, G.D. (2010) *Curr Opin Struct Biol* 20, 73-81.
(5) Gangaraju, V.K. and Bartholomew, B. (2007) *Mutat Res* 618, 3-17.
(6) Lessard, J.A. and Crabtree, G.R. (2010) *Annu Rev Cell Dev Biol* 26, 503-32.
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(9) Simone, C. (2006) *J Cell Physiol* 207, 309-14.
(10) Yamamichi, N. et al. (2005) *Oncogene* 24, 5471-81.
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(12) Shen, H. et al. (2008) *Cancer Res* 68, 10154-62.
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(15) Gao, X. et al. (2008) *Proc Natl Acad Sci U S A* 105, 6656-61.
(16) Han, D. et al. (2008) *Dev Biol* 315, 136-46.
(17) Kim, J.K. et al. (2001) *Mol Cell Biol* 21, 7787-95.
(18) Schaniel, C. et al. (2009) *Stem Cells* 27, 2979-91.
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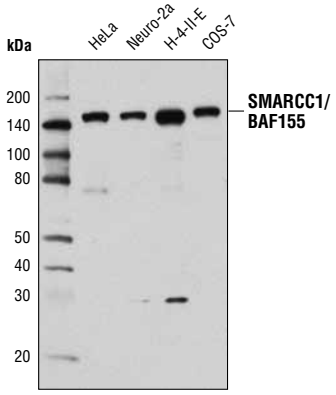
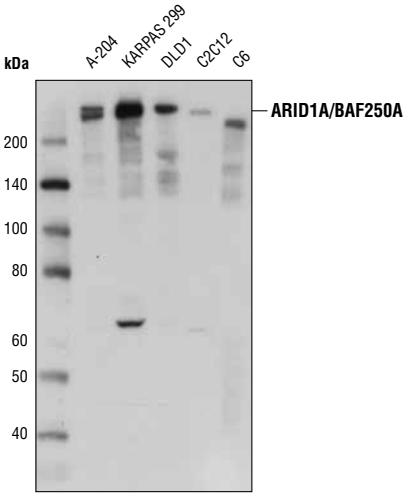
(21) Ho, L. et al. (2009) *Proc Natl Acad Sci USA* 106, 5181-6.

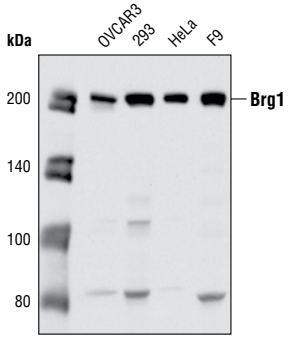
Applications Key: **W**—Western **IP**—Immunoprecipitation **IHC**—Immunohistochemistry **ChIP**—Chromatin Immunoprecipitation **IF**—Immunofluorescence **F**—Flow cytometry **E-P**—ELISA-Peptide
Species Cross-Reactivity Key: **H**—human **M**—mouse **R**—rat **Hm**—hamster **Mk**—monkey **Mi**—mink **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.



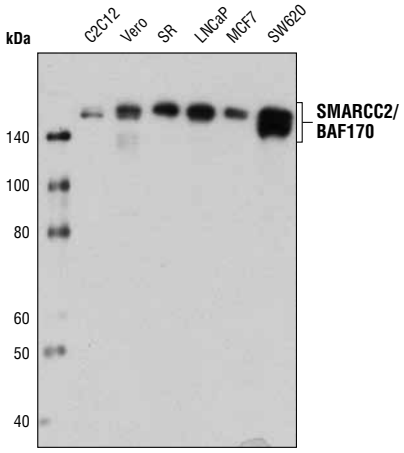
Western blot analysis of extracts from various cell lines using ***SMARCC1/BAF155 (D7F8S) Rabbit mAb #11956****.*



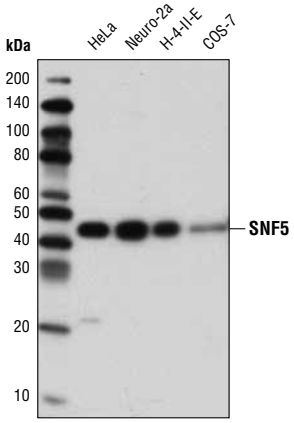
Western blot analysis of extracts from various cell lines using ***ARID1A/BAF250A (D2A8U) Rabbit mAb #12354****.*



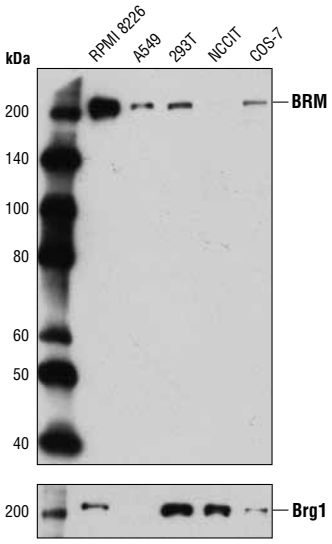
Western blot analysis of extracts of various cell lines using ***Brg1 (A52) Antibody #3508****.*



Western blot analysis of extracts from various cell lines using ***SMARCC2/BAF170 (D8O9V) Rabbit mAb #12760****.*



Western blot analysis of extracts from various cell lines using ***SNF5 (D9C2) Rabbit mAb #8745****.*



Western blot analysis of extracts from various cell lines using ***BRM (D9E8B) XP® Rabbit mAb #11966*** *(upper) or Brg1 (A52) Antibody #3508 (lower).*

#12854

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.