#12755 Store at -20°C

High Mobility Group (HMG) Proteins Antibody Sampler Kit

☑ 1 Kit
(5 x 40 µl)

Cell Signaling TECHNOLOGY®

Orders ■ 877-616-CELL (2355)
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Web ■ www.cellsignal.com

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

Products Included	Product #	Quantity	Mol. Wt.	Isotype
HMGA1 (D6A4) XP® Rabbit mAb	7777	40 µl	18 kDa	Rabbit IgG
HMGB1 (D3E5) Rabbit mAb	6893	40 µl	29 kDa	Rabbit IgG
HMBGB2 (D1P9V) Rabbit mAb	14163	40 µl	28 kDa	Rabbit IgG
HMGN1 (D1I5O) Rabbit mAb	12734	40 µl	18 kDa	Rabbit IgG
HMGN2 (D9B9) XP® Rabbit mAb	9437	40 µl	17 kDa	Rabbit IgG
Anti-rabbit IgG, HRP-linked Antibody	7074	100 µl		Goat

See www.cellsignal.com for individual companion 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 antibody.*

Recommended Antibody Dilutions:

Western blotting	1:1000

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

Description: The High Mobility Group (HMG) Proteins Antibody Sampler Kit provides an economical means of detecting total protein from the HMG family members including HMGA1, HMGB1, HMGB2, HMGN1 and HMGN2. The kit contains enough primary antibody to perform four western blots per primary antibody.

Background: High mobility group (HMG) proteins are a superfamily of abundant and ubiquitous nuclear proteins that bind DNA without sequence specificity and induce structural changes to the chromatin fiber to regulate access to the underlying DNA (1). HMGA1, formerly known as HMG-I/Y, belongs to a family of high mobility group proteins known as HMGA. HMGA proteins are considered architectural transcription factors; they do not have direct transcriptional activation capacity, but instead regulate gene expression by changing DNA conformation through binding to AT-rich regions in the DNA and/or direct interaction with other transcription factors (2). HMGA1 is highly expressed during embryogenesis and in embryonic stem cells, but not in fully differentiated adult tissues (3,4). High mobility group protein B1 (HMGB1) and high mobility group protein B2 (HMGB2) belong to a family of highly conserved proteins that contain HMG box domains (5). HMGB1 is a widely expressed and highly abundant protein (6). HMGB2 is widely expressed during embryonic development, but it is restricted to lymphoid organs and testis in adult animals (7). While expression varies, the biochemical properties of the different family members may be indistinguishable. HMGB proteins are recruited by and help facilitate the assembly of site-specific DNA binding proteins to their cognate binding sites in chromatin. For example, HMGB1 and HMGB2 facilitate the binding of Hox proteins, Oct proteins, p53, Rel proteins, and steroid hormone receptor proteins to their target gene promoters (5,6). In addition to their functions in the nucleus, HMGB proteins play a significant role in extracellular signaling associated with inflammation. HMGB1 is massively released into the extracellular environment during cell necrosis, but not apoptosis. Extracellular HMGB1 "alarms" the innate immune system by acting as a chemoattractant for inflammatory cells triggering activation of T cells and dendritic cells. In addition, activated monocytes, macrophages, and dendritic cells also secrete HMGB1 (6). HMGB2 is secreted by myeloid cells and promotes proliferation and migration of endothelial cells by binding to the receptor for advanced glycation end products (RAGE) (8). The HMGN family of proteins, which includes five members (HMGN1-5) (1) function in transcriptional regulation and are recruited to gene promoters by transcription factors, such as estrogen receptor α (ERα), serum responsive factor (SRF), and PITX2, where they can facilitate either gene activation or repression (9-11). The expression of HMGN1 (also known as HMG14) and HMGN2 (also known as HMG17) is tightly linked to cellular differentiation. HMGN1 and HMNG2 are ubiquitous and highly expressed in all embryonic tissues. During mouse embryogenesis, expression is down-regulated throughout the embryo, except in committed but continuously renewing cell types undergoing active differentiation, such as the basal layer of the epithelium and kidney cells undergoing mesenchyme to epithelium transition (12,13).

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. HMGA1 (D6A4) XP® Rabbit mAb recognizes isoforms 1a and 1b.

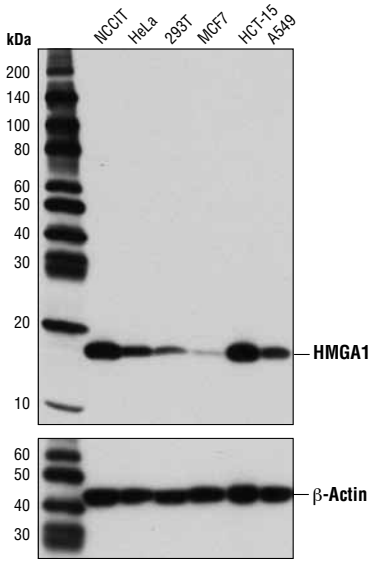
Source/Purification: Monoclonal antibody is produced by immunizing animals with synthetic peptides corresponding to residues surrounding Gly68 of human HMGA1, Ala137 of Human HMGB1, Val32 of human HMGN1, Glu169 of human HMGB2 protein or Asp74 of human HMGN2 protein.

Background References:

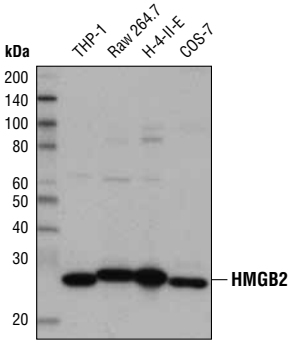
(1) Hock, R. et al. (2007) *Trends Cell Biol* 17, 72-9.
(2) Cleynen, I. and Van de Ven, W.J. (2008) *Int J Oncol* 32, 289-305.
(3) Chiappetta, G. et al. (1996) *Oncogene* 13, 2439-46.
(4) Ben-Porath, I. et al. (2008) *Nat Genet* 40, 499-507.
(5) Thomas, J.O. and Travers, A.A. (2001) *Trends Biochem Sci* 26, 167-74.
(6) Müller, S. et al. (2004) *J Intern Med* 255, 332-43.
(7) Ronfani, L. et al. (2001) *Development* 128, 1265-73.
(8) Pusterla, T. et al. (2009) *Autoimmunity* 42, 308-10.
(9) Zhu, N. and Hansen, U. (2007) *Mol Cell Biol* 27, 8859-73.
(10) Amen, M. et al. (2008) *Nucleic Acids Res* 36, 462-76.
(11) Belova, G.I. et al. (2008) *J Biol Chem* 283, 8080-8.
(12) Furusawa, T. et al. (2006) *Mol Cell Biol* 26, 592-604.
(13) Lehtonen, S. and Lehtonen, E. (2001) *Differentiation* 67, 154-63.

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

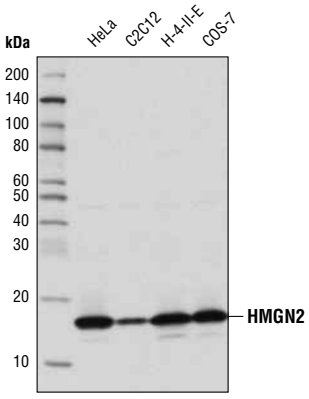
Species Cross-Reactivity Key: **H**—human **M**—mouse **R**—rat **Hm**—hamster **Mk**—monkey **Mi**—mink **C**—chicken **Dm**—D. melanogaster **X**—Xenopus **Z**—zebrafish **B**—bovine **Dg**—dog **Pg**—pig **Sc**—S. cerevisiae **All**—all species expected Species enclosed in parentheses are predicted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



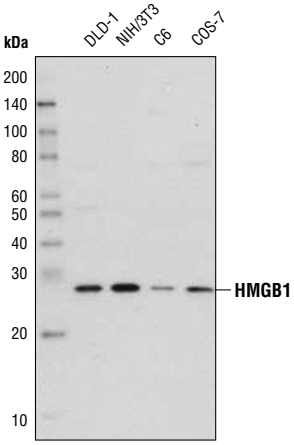
Western blot analysis of extracts from various cell lines using ***HMGA1 (D6A4) XP® Rabbit mAb #7777*** *(upper) or β-Actin (D6A8) Rabbit mAb #8457 (lower).*



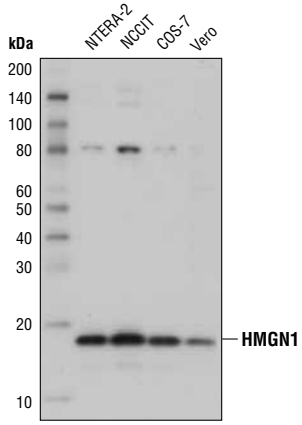
Western blot analysis of extracts from various cell lines using ***HMGB2 (D1P9V) Rabbit mAb #14163****.*



Western blot analysis of extracts from various cell lines using ***HMGN2 (D9B9) XP® Rabbit mAb #9437****.*



Western blot analysis of extracts from various cell lines using ***HMGB1 (D3E5) Rabbit mAb #6893****.*



Western blot analysis of extracts from various cell lines using ***HMGN1 (D1I5O) Rabbit mAb #12734****.*

#12755

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.