#8614 Store at -20°C

Cytoskeletal Marker Antibody Sampler Kit

☑ 1 Kit
(6 x 20 µl)

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
β-Actin (D6A8) Rabbit mAb	8457	20 µl	45 kDa	Rabbit IgG
Desmin (D93F5) XP® Rabbit mAb	5332	20 µl	53 kDa	Rabbit IgG
Keratin 17 (D73C7) Rabbit mAb	4543	20 µl	48 kDa	Rabbit IgG
Pan-Keratin (C11) Mouse mAb	4545	20 µl	46–62 kDa	Mouse IgG1
β-Tubulin (9F3) Rabbit mAb	2128	20 µl	55 kDa	Rabbit IgG
Vimentin (D21H3) XP® Rabbit mAb	5741	20 µl	57 kDa	Rabbit IgG
Anti-rabbit IgG, HRP-linked Antibody	7074	100 µl		Goat
Anti-mouse IgG, HRP-linked Antibody	7076	100 µl		Horse

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 antibody.*

Recommended Antibody Dilutions:

Western blotting	1:1000

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

Description: The Cytoskeletal Marker Antibody Sampler Kit provides an economical means to evaluate the presence and status of select cytoskeleton associated proteins. The kit includes enough antibody to perform two western blot experiments for each primary antibody.

Background: The cytoskeleton consists of three different types of cytosolic fibers: microtubules, microfilaments (actin) and intermediate filaments. Actin, a ubiquitous eukaryotic protein, is the major component of the cytoskeleton. At least six isoforms are known in mammals. Nonmuscle β- and γ-actin, also known as cytoplasmic actin, are predominantly expressed in nonmuscle cells, controlling cell structure and motility (1). Major types of intermediate filaments are distinguished in part by the tissue in which they are expressed, for example; cytokeratins (epithelial cells), vimentin (mesenchyme origin), and desmin (skeletal, visceral and certain vascular smooth muscle cells) (2). Keratin heterodimers composed of an acidic keratin (or type I keratin, keratins 9 to 23) and a basic keratin (or type II keratin, keratins 1 to 8) assemble to form intermediate filaments (3). Research studies have demonstrated that vimentin is present in sarcomas, but not carcinomas, and its expression is examined relative to other markers in order to distinguish between the two forms of neoplasm (4). Desmin is a myogenic marker expressed in early development that forms a network of filaments that extends across the myofibril and surrounds Z discs (5). α/β-tubulin heterodimers form the tubulin subunit that comprises the microtubule building block (6).

Specificity/Sensitivity: Desmin (D93F5) XP® Rabbit mAb, Keratin 17 (D73C7) Rabbit mAb, β-Tubulin (9F3) Rabbit mAb, Vimentin (D21H3) XP® Rabbit mAb, and β-Actin (D6A8) Rabbit mAb recognize endogenous levels of total respective target proteins. Pan-Keratin (C11) Mouse mAb detects endogenous levels of total keratins 4, 5, 6, 8, 10, 13, and 18. This antibody does not cross-react with other keratins.

Source/Purification: Rabbit monoclonal antibodies are produced by immunizing animals with a synthetic peptide cooresponding to residues near the amino terminus of human β-actin protein, carboxy terminal residues of human desmin protein, residues near the carboxy terminus of human keratin 17 protein, the amino terminus of human β-tubulin protein, or residues surrounding Arg45 of human vimentin protein. Mouse monoclonal antibody is produced by immunizing animals with a cytoskeleton preparation from A-431 cells.

Background References:

(1) Herman, I.M. (1993) *Curr Opin Cell Biol* 5, 48-55.

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

(3) Moll, R. et al. (1982) *Cell* 31, 11-24.

(4) Leader, M. et al. (1987) *Histopathology* 11, 63-72.

(5) Li, Z. et al. (1996) *Dev Biol* 175, 362-6.

(6) Westermann, S. and Weber, K. (2003) *Nat Rev Mol Cell Biol* 4, 938-47.

U.S. Patent No. 5,675,063

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 **Ce**—C. elegans **Hr**—horse **All**—all species expected Species enclosed in parentheses are predicted to react based on 100% homology.

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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.