

Cytokeratin Antibody Sampler Kit



✓ 1 Kit
(6 x 40 µl)

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rev. 07/15/09

This product is for *in vitro* research use only and is not intended for use in humans or animals.
This product is not intended for use as a therapeutic or in diagnostic procedures.

Products Included	Product #	Quantity	Mol. Wt.	Isotype
Keratin 7 Antibody	3473	40 µl	52 kDa	Rabbit IgG
Keratin 17 (D73C7) XP™ Rabbit mAb	4543	40 µl	48 kDa	Rabbit IgG
Keratin 18 (DC10) Mouse mAb	4548	40 µl	46 kDa	Mouse IgG1
Keratin 8/18 (C51) Mouse mAb	4546	40 µl	46, 55 kDa	Mouse IgG1
Keratin 19 (BA17) Mouse mAb	4558	40 µl	40 kDa	Mouse IgG1
Pan-Keratin (C11) Mouse mAb	4545	40 µl	46, 55 kDa	Mouse IgG1
Anti-mouse IgG, HRP-linked Antibody	7076	100 µl		Horse
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 Cytokeratin Antibody Sampler Kit provides an economical means to evaluate the presence and status of selected keratin proteins. The kit provides enough primary and secondary antibodies to perform four western mini-blot experiments per primary antibody.

Background: Keratins (cytokeratins) are intermediate filament proteins that are mainly expressed in epithelial cells. Keratins 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 filaments (1,2). Keratin isoforms demonstrate tissue- and differentiation-specific profiles that make them useful as biomarkers (1). Mutations in keratin genes are associated with skin disorders, liver and pancreatic diseases, and inflammatory intestinal diseases (3-6).

Specificity/Sensitivity: The Pan-Keratin (C11) mouse mAb detects endogenous levels of total keratins 4, 5, 6, 8, 10, 13 and 18. The antibody does not cross-react with other keratins. Each of the remaining antibodies included in this kit detect endogenous levels of the specified keratin protein and do not cross-react with other keratin proteins.

Source/Purification: Pan-Keratin Mouse mAb (C11) is produced by immunizing animals with a cytoskeleton preparation from A431 cells. Keratin 8/18 (C51) Mouse mAb is produced by immunizing animals with a cytoskeleton preparation from HeLa cells. Keratin 18 (DC10) Mouse mAb is produced by immunizing animals with human PMC-42 breast carcinoma cells. Keratin 19 (BA17) Mouse mAb is produced by immunizing animals with detergent-insoluble extract of human mammary epithelial organoids. Keratin 7 Antibody is generated by immunizing animals with a synthetic peptide (KLH-coupled) corresponding to amino acids at the amino terminus of human keratin 7. Keratin 17 (D73C7) XP™ Rabbit mAb is produced by immunizing

animals with a synthetic peptide (KLH-coupled) corresponding to amino acids near the carboxy terminus of human keratin 17. Polyclonal antibodies are purified by Protein A and peptide

Background References:

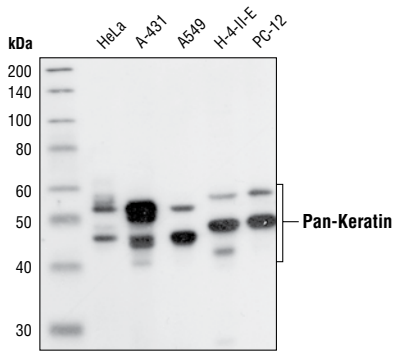
- (1) Moll, R. et al. (1982) *Cell* 31, 11–24.
- (2) Chang, L. and Goldman, R.D. (2004) *Nat. Rev. Mol. Cell Biol.* 5, 601–613.
- (3) Ramaekers, F.C. and Bosman, F.T. (2004) *J. Pathol.* 204, 351–354.
- (4) Lane, E.B. and McLean, W.H. (2004) *J. Pathol.* 204, 355–366.
- (5) Zatloukal, K. et al. (2004) *J. Pathol.* 204, 367–376.
- (6) Owens, D.W. and Lane, E.B. (2004) *J. Pathol.* 204, 377–385.

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.

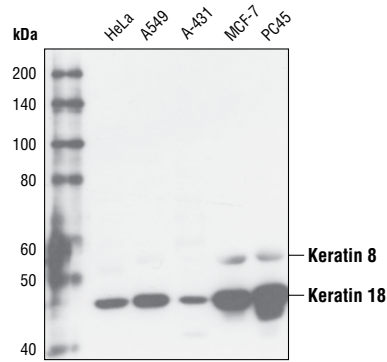
Recommended Antibody Dilutions:

Western blotting 1:1000
Keratin 18 (DC10) Mouse mAb #4548
Western blotting 1:2000

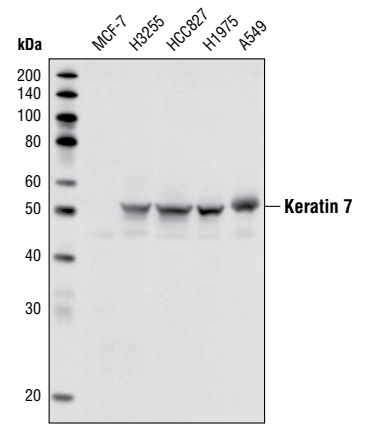
Please visit www.cellsignal.com for a complete listing of recommended companion products.



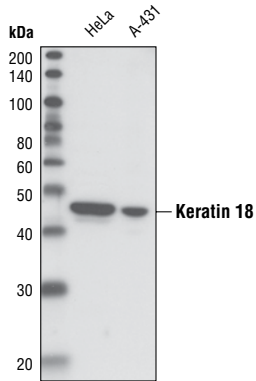
Western blot analysis of extracts from various cell lines using **Pan-Keratin (C11) Mouse mAb #4545**.



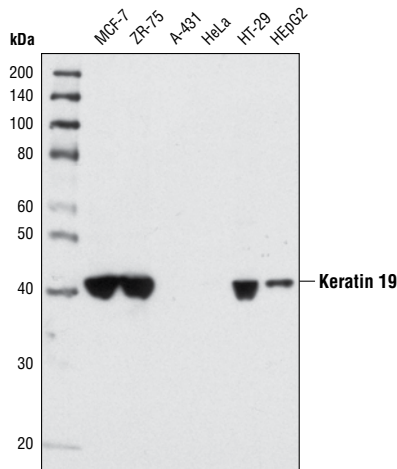
Western blot analysis of extracts from various cell lines using **Keratin 8/18 (C51) Mouse mAb #4546**.



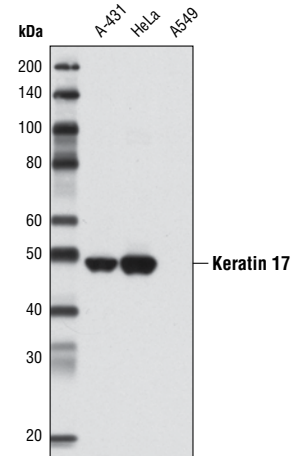
Western blot analyses of extracts of various cell lines using **Keratin 7 Antibody #3473**. As expected, keratin 7 is absent in MCF-7 cells.



Western blot analysis of extracts from HeLa and A431 cells, using **Keratin 18 (DC10) Mouse mAb #4548**.



Western blot analysis of extracts from various cell types using **Keratin 19 (BA17) Mouse mAb #4558**. As expected, the protein is absent in A-431 and HeLa cells.



Western blot analysis of extracts from A-431, HeLa, and A549 cells using **Keratin 17 (D73C7) XP™ Rabbit mAb #4543**. As expected, keratin 17 is absent in A549 cells.

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.

- 1X Phosphate Buffered Saline (PBS)
- 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
- Transfer Buffer:** 25 mM Tris base, 0.2 M glycine, 20% methanol (pH 8.5)
- 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).
- Nonfat Dry Milk (weight to volume [w/v])
- 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%).
- Wash Buffer:** 1X TBS, 0.1% Tween-20 (TBS/T)
- Bovine Serum Albumin (BSA)
- 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%).
- 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.
- Prestained Protein Marker, Broad Range (Premixed Format) #7720
- Biotinylated Protein Ladder Detection Pack #7727
- 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.

- Treat cells by adding fresh media containing regulator for desired time.
- Aspirate media from cultures; wash cells with 1X PBS; aspirate.
- 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.
- Sonicate for 10–15 seconds to shear DNA and reduce sample viscosity.
- Heat a 20 µl sample to 95–100°C for 5 minutes; cool on ice.
- Microcentrifuge for 5 minutes.
- 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.

- Electrotransfer to nitrocellulose or PVDF membrane.

C Membrane Blocking and Antibody Incubations

NOTE: Volumes are for 10 cm x 10 cm (100 cm²) of membrane; for different sized membranes, adjust volumes accordingly.

- (Optional) After transfer, wash nitrocellulose membrane with 25 ml TBS for 5 minutes at room temperature.
- Incubate membrane in 25 ml of blocking buffer for 1 hour at room temperature.
- Wash three times for 5 minutes each with 15 ml of TBS/T.
- Incubate membrane and primary antibody (at the appropriate dilution) in 10 ml primary antibody dilution buffer with gentle agitation overnight at 4°C.
- Wash three times for 5 minutes each with 15 ml of TBS/T.
- 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.
- Wash three times for 5 minutes each with 15 ml of TBS/T.

D Detection of Proteins

- 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.

- 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.