Store at -20°C

#8678

Oncogene and Tumor Suppressor Antibody Sampler Kit

☑ 1 Kit
(8 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
Phospho-Akt (Ser473) (D9E) XP® Rabbit mAb	4060	20 µl	60 kDa	Rabbit IgG
BRCA1 Antibody	9010	20 µl	220 kDa	Rabbit IgG
E-Cadherin (24E10) Rabbit mAb	3195	20 µl	135 kDa	Rabbit IgG
Phospho-Estrogen Receptor α (Ser167) (D1A3) Rabbit mAb	5587	20 µl	66 kDa	Rabbit IgG
HER2/ErbB2 (D8F12) XP® Rabbit mAb	4290	20 µl	185 kDa	Rabbit IgG
p53 (7F5) Rabbit mAb	2527	20 µl	53 kDa	Rabbit IgG
PTEN (138G6) Rabbit mAb	9559	20 µl	54 kDa	Rabbit IgG
Stathmin Antibody	3352	20 µl	19 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.

Storage: Supplied in 136 mM NaCl, 2.6 mM KCl, 12 mM sodium phosphate (pH 7.4) dibasic, 2 mg/ml BSA, and 50% glycerol. *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 Oncogenes and Tumor Suppressor Antibody Sampler Kit offers an economical means of investigating proteins commonly involved in the biological pathways behind oncogenesis, tumor metastasis, and cancer pathology. The kit includes antibody to perform two western blot experiments with each antibody.

Background: Oncogenesis is a multistep process leading to sequential alterations in several oncogenes, tumor-suppressor genes, and microRNA genes (1,2). These alterations often disrupt the expression, function, and/or activity of proteins regulating cell growth and programmed cell death. Many of the molecular mechanisms and biological pathways driving oncogenesis and cancer pathology have been identified. The signal transduction pathways regulating apoptosis, cell-cycle progression, cell adhesion, cell migration, and DNA damage responses are often disrupted. HER2/ErbB2 (3), E-Cadherin (4), p53 (5,6), Stathmin (7), BRCA1 (8,9), Akt (10), PTEN (11), and Estrogen Receptor α (12) function in many of these pathways.

Specificity/Sensitivity: Phospho-Akt (Ser473) (D9E) XP® Rabbit mAb recognizes endogenous levels of Akt only when phosphorylated at Ser473. BRCA1 Antibody recognizes endogenous levels of total BRCA1 protein. The antibody detects BRAC1 nuclear isoforms 1, 2, and 4, but not BRAC1 cytoplasmic isoforms 3 and 5, and does not detect BRCA2. E-Cadherin (24E10) Rabbit mAb recognizes endogenous levels of total E-cadherin protein. The antibody does not cross-react with related family members, such as N-cadherin. Phospho-Estrogen Receptor α (Ser167) (D1A3) Rabbit mAb recognizes endogeneous levels of ERα only when phosphorylated at Ser167. The antibody cross reacts with a nonspecific band at around 77 kDa. HER2/ErbB2 (D8F12) XP® Rabbit mAb recognizes endogenous levels of total HER2/ErbB2 protein. p53 (7F5) Rabbit mAb detects endogenous levels of total p53 protein. The antibody binding has been mapped to the amino terminus region of human p53 protein. PTEN (138G6) Rabbit mAb recognizes endogenous levels of total PTEN protein. Stathmin Antibody recognizes endogenous levels of total stathmin protein. The antibody does not cross-react with related proteins, such as SCG10, SCLIP, and RB3.

Source/Purification: Phospho-specific monoclonal antibodies are produced by immunizing animals with a synthetic phosphopeptide corresponding to residues surrounding Ser473 of human Akt protein or Ser167 of human estrogen receptor α protein. Monoclonal antibodies are produced by immunizing animals with a synthetic peptide corresponding to residues surrounding Pro780 of human E-cadherin protein, residues near the amino terminus of human HER2/ErbB2 protein, the carboxy terminus of human PTEN protein, or with a full-length human p53 fusion protein. Polyclonal antibodies are produced by immunizing animals with a synthetic peptide corresponding to residues surrounding the amino terminus of human BRCA1 protein or residues surrounding Ser38 of human stathmin protein. Polyclonal antibodies are purified by protein A and peptide affinity chromatography.

Background References:

(1) Berger, A.H. et al. (2011) *Nature* 476, 163-9.
(2) Peltomäki, P. (2012) *Exp Cell Res* 318, 299-310.
(3) Dittadi, R. and Gion, M. (2000) *J Natl Cancer Inst* 92, 1443-4.
(4) Hazan, R.B. et al. (2004) *Ann N Y Acad Sci* 1014, 155-63.
(5) Rahman, N. and Stratton, M.R. (1998) *Annu Rev Genet* 32, 95-121.
(6) Freed-Pastor, W.A. and Prives, C. (2012) *Genes Dev* 26, 1268-86.
(7) Belletti, B. and Baldassarre, G. (2011) *Expert Opin Ther Targets* 15, 1249-66.
(8) Gayther, S.A. et al. (1999) *Am J Hum Genet* 65, 1021-9.
(9) Scully, R. and Livingston, D.M. (2000) *Nature* 408, 429-32.
(10) Jazirehi, A.R. et al. (2012) *Am J Cancer Res* 2, 178-91.
(11) Saal, L.H. et al. (2008) *Nat Genet* 40, 102-7.
(12) Pópulo, H. et al. (2012) *Int J Mol Sci* 13, 1886-918.

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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 (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.

1. 1X Phosphate Buffered Saline (PBS)
2. **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
3. **Transfer Buffer:** 25 mM Tris base, 0.2 M glycine, 20% methanol (pH 8.5)
4. **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).
5. Nonfat Dry Milk (weight to volume [w/v])
6. **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%).
7. **Wash Buffer:** 1X TBS, 0.1% Tween®20 (TBS/T)
8. Bovine Serum Albumin (BSA)
9. **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%).
10. **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.
11. Prestained Protein Marker, Broad Range (Premixed Format) #7720
12. Biotinylated Protein Ladder Detection Pack #7727
13. **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.

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 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.
4. Sonicate for 10–15 seconds to shear DNA and reduce sample viscosity.
5. Heat a 20 µl sample to 95–100°C for 5 minutes; cool on ice.
6. Microcentrifuge for 5 minutes.
7. 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.

8. Electrotransfer to nitrocellulose or PVDF membrane.

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.

1. (Optional) After transfer, wash nitrocellulose membrane with 25 ml TBS for 5 minutes at room temperature.
2. Incubate membrane in 25 ml of blocking buffer for 1 hour at room temperature.
3. Wash three times for 5 minutes each with 15 ml of TBS/T.
4. Incubate membrane and primary antibody (at the appropriate dilution) in 10 ml primary antibody dilution buffer with gentle agitation overnight at 4°C.
5. Wash three times for 5 minutes each with 15 ml of TBS/T.
6. 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.
7. Wash three times for 5 minutes each with 15 ml of TBS/T.

D Detection of Proteins

1. 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.

2. 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.

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