Store at -20°C

#5142

Loading Control Antibody Sampler Kit

☑ 1 Kit
(5 x 20 µl)

Cell Signaling TECHNOLOGY®

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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
COX IV (3E11) Rabbit mAb	4850	20 µl	17 kDa	Rabbit IgG
GAPDH (D16H11) XP® Rabbit mAb	5174	20 µl	37 kDa	Rabbit IgG
Histone H3 (D1H2) XP® Rabbit mAb	4499	20 µl	17 kDa	Rabbit IgG
β-Tubulin (9F3) Rabbit mAb	2128	20 µl	55 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 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 validation data and a complete listing of recommended companion products.

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

Description: The Loading Control Antibody Sampler Kit contains antibodies to a variety of housekeeping proteins. The kit includes enough antibody to perform two western blots experiments with each primary antibody.

Background: Housekeeping proteins perform numerous basic functions within the cell and are constitutively expressed at high levels in a variety of tissues and cell types. Western blot analysis commonly uses housekeeping proteins such as β-actin, COX IV, GAPDH, histone H3 and the α- and β-tubulins as loading controls. Actin is a ubiquitous protein and a major component of the eukaryotic cytoskeleton. Actin exists mainly as the F-actin fibrous polymer (1). Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) catalyzes the phosphorylation of glyceraldehyde-3-phosphate during glycolysis. Recent work has demonstrated that GAPDH plays roles in apoptosis (2), gene expression (3), and nuclear transport (4). Globular tubulin subunits made up of α- and β-tubulin heterodimers are the building blocks of microtubules, one of three types of cytosolic fibers that comprise the cytoskeleton (5). Histone proteins, including histone H3, make up the primary building block of chromatin known as nucleosomes. Modulation of the chromatin structure plays an important role in the regulation of transcription in eukaryotes (6). Cytochrome c oxidase (COX) is a hetero-oligomeric enzyme consisting of 13 subunits localized to the inner mitochondrial membrane (7-9). It is the terminal enzyme complex in the respiratory chain, catalyzing the reduction of protons across the mitochondrial inner membrane to drive ATP synthesis (10).

Specificity/Sensitivity: Each antibody in the Loading Control Antibody Sampler Kit detects endogenous levels of its target protein and does not typically cross-react with other proteins.

Source/Purification: Monoclonal antibody is produced by immunizing animals with synthetic peptides corresponding to residues near the amino terminus of human β-actin, surrounding Lys29 of human COX IV, the sequence of human GAPDH, and the carboxy-terminal residues of human histone H3.

Background References:

(1) Condeelis, J. (2001) *Trends Cell Biol* 11, 288–93.
(2) Hara, M.R. and Snyder, S.H. *Cell Mol Neurobiol* 26, 527–38.
(3) Zheng, L. et al. (2003) *Cell* 114, 255–66.
(4) Bae, B.I. et al. (2006) *Proc Natl Acad Sci U S A* 103, 3405–9.
(5) Westermann, S. and Weber, K. (2003) *Nat Rev Mol Cell Biol* 4, 938–47.
(6) Workman, J.L. and Kingston, R.E. (1998) *Annu Rev Biochem* 67, 545–79.
(7) Ostermeier, C. et al. (1996) *Curr Opin Struct Biol* 6, 460–6.
(8) Capaldi, R.A. et al. (1983) *Biochim Biophys Acta* 726, 135–48.
(9) Kadenbach, B. et al. (2000) *Free Radic Biol Med* 29, 211–21.
(10) Barrientos, A. et al. (2002) *Gene* 286, 53–63.

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.

#5142

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.