Store at -20°C

#9962

# Epitope Tag Antibody Sampler Kit

1 Kit
(5 x 40 µl)

Cell Signaling TECHNOLOGY®

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

| Products Included | Product # | Quantity | Isotype |
|---|---|---|---|
| Myc-Tag (71D10) Rabbit mAb | 2278 | 20 µl | Rabbit IgG |
| HA-Tag (C29F4) Rabbit mAb | 3724 | 20 µl | Rabbit IgG |
| GST-Tag (91G1) Rabbit mAb | 2625 | 20 µl | Rabbit IgG |
| DYKDDDDK Tag (9A3) Mouse mAb (Binds to same epitope as Sigma's Anti-FLAG® M2 Antibody) | 8146 | 20 µl | Mouse IgG1 |
| His-Tag (D3I1O) XP® Rabbit mAb | 12698 | 20 µl | 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.**

**Description:** The Epitope Tag Antibody Sampler Kit provides an economical means to analyze the expression of a variety of epitope tagged proteins. The kit includes enough antibody to perform two western blot experiments with each primary antibody.

**Background:** Epitope tags are useful for the labeling and detection of proteins using immunoblotting, immunoprecipitation and immunostaining techniques. Due to their small size, they are unlikely to affect the tagged protein's biochemical properties.

Several different epitope tags are now commonly utilized and readily available. For instance, a variety of plasmids contain DNA that encodes an amino-terminal tag consisting of six histidine (6xHis) residues followed by an extended multiple cloning site. The 6xHis tag on the expressed recombinant proteins allows for efficient coupling to Ni2+ affinity resins and purification by single step chromatography (1). As is the case with other protein tag systems (2), this polyhistidine tag can often be cleaved at sites recognized by proteases such as thrombin and enterokinases to isolate the protein of interest (1). Glutathione S-transferase (GST) is another widely used fusion partner, since it provides both an easily detectable Tag and a simple purification process with little effect on the biological function of the protein of interest. Numerous vectors containing GST-Tag have been developed for both prokaryotic and eukaryotic systems over the past decade (3-5). The HA tag, derived from an epitope of the influenza hemagglutinin protein, has also been extensively used as a general epitope tag in expression vectors (6), while the Myc epitope tag is routinely used to detect expression of recombinant proteins in bacteria, yeast, insect and mammalian cell systems (7). Finally, the DYKDDDDK peptide has been used extensively as a general epitope tag in expression vectors and consists of only eight amino acids. This peptide can be expressed and detected with the protein of interested as an amino-terminal or carboxy-terminal fusion (8).

**Specificity/Sensitivity:** All antibodies in the Epitope Tag Antibody Sampler Kit detect overexpressed fusion proteins containing the corresponding epitope tags. DYKDDDDK Tag Antibody recognizes the DYKDDDDK peptide (the same epitope recognized by Sigma's Anti-FLAG® antibodies), and its binding specificity is NOT dependent on the presence of divalent metal cations.

**Source/Purification:** Rabbit monoclonal antibodies are produced by immunizing rabbits with a GST fusion protein, a synthetic peptide corresponding to residues 410-419 of human c-Myc (EQKLISEEDL), residues of the 6xHis epitope tag, or with a synthetic peptide containing the influenza hemagglutinin epitope (YPYDVPDYA). Mouse monoclonal antibodies are produced by immunizing animals with a synthetic DYKDDDDK peptide.

**Storage:** Supplied in 10 mM sodium HEPES (pH 7.5), 150 mM NaCl, 100 µg/ml BSA and 50% glycerol. Store at –20°C. *Do not aliquot the antibodies.*

**Recommended Antibody Dilutions:**

| | |
|---|---|
| Western blotting | 1:1000 |

**#8146: please use 5% nonfat dry milk for primary antibody incubation.**

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

**Background References:**

(1) Kroll, D.J. et al. (1993) *DNA Cell Biol.* 12, 441–453.
(2) di Guan, C. et al. (1988) *Gene* 67, 21–30.
(3) Guan, K.L. and Dixon, J.E. (1991) *Anal. Biochem.* 192, 262–267.
(4) Davis, A.H. et al. (1993) *Biotechnology* 11, 933–936.
(5) Yu, J. et al. (1998) *Mol. Cell. Biol.* 18, 1379–1387.
(6) Field, J. et al. (1988) *Mol. Cell. Biol.* 8, 2159–2165.
(7) Munro, S. and Pelham, H.R. (1984) *EMBO J.* 3, 3087–3093.
(8) Brizzard, B.L. et al. (1994) *Biotechniques* 16, 730–735.





**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% sequence 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.