#12866 Store at -20°C

Glycolysis II Antibody Sampler Kit

☑ 1 Kit
(8 x 40 µl)

Cell Signaling TECHNOLOGY®

Orders ■ 877-616-CELL (2355)
orders@cellsignal.com
Support ■ 877-678-TECH (8324)
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For Research Use Only. Not For Use In Diagnostic Procedures.

Products Included	Product #	Quantity	Mol. Wt.	Isotype
Aldolase A (D73H4) Rabbit mAb	8060	40 µl	40 kDa	Rabbit IgG
Enolase-1 Antibody	3810	40 µl	47 kDa	Rabbit IgG
Enolase-2 (D20H2) Rabbit mAb	8171	40 µl	47 kDa	Rabbit IgG
PDHK1 (C47H1) Rabbit mAb	3820	40 µl	47 kDa	Rabbit IgG
PFKFB2 (D7G5R) Rabbit mAb	13045	40 µl	55 kDa	Rabbit IgG
PFKFB3 (D7H4Q) Rabbit mAb	13123	40 µl	60 kDa	Rabbit IgG
PFKL Antibody	8175	40 µl	78 kDa	Rabbit IgG
PGAM1 (D3J9T) Rabbit mAb	12098	40 µl	28 kDa	Rabbit IgG
Anti-rabbit IgG, HRP-linked Antibody	7074	100 µl		Goat

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 a complete listing of recommended companion products.

See www.cellsignal.com for individual component applications, species cross-reactivity, dilutions and additional application protocols.

Description: The Glycolysis II Antibody Sampler Kit provides an economical means to investigate select enzymes involved in glycolysis. The kit contains enough primary antibody to perform four western blot experiments per primary antibody.

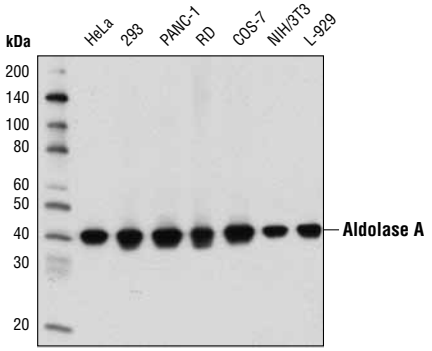
Background: Glycolysis is the metabolic process by which glucose is converted to pyruvate in a sequence of enzymatic steps. Phosphofructokinase (PFK) catalyzes the phosphorylation of fructose-6-phosphate in glycolysis (1). The bifunctional 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase (PFK-2/FBPase or PFKFB) catalyzes the synthesis and degradation of fructose 2,6-bisphosphate and regulates its steady-state level. Four different PFKFB isoforms (PFKFB1, PFKFB2, PFKFB3, and PFKFB4) have been identified (2). Aldolase (fructose bisphosphate aldolase) is a glycolytic enzyme that catalyzes the conversion of fructose 1, 6-bisphosphate to 3-phosphoglyceraldehyde (3). Phosphoglycerate mutase (PGAM1) catalyzes the conversion of 3-phosphoglycerate to 2-phosphoglycerate during glycolysis (4). Enolase is an important glycolytic enzyme involved in the interconversion of 2-phosphoglycerate to phosphoenolpyruvate. Mammalian enolase exists as three subunits: enolase-1 (α-enolase), enolase-2 (γ-enolase) and enolase-3 (β-enolase) that can form both homo- and heterodimers (5). Pyruvate dehydrogenase kinase (PDHK) phosphorylates PDH and inactivates it, whereas dephosphorylation of PDH is carried out by pyruvate dehydrogenase phosphatase to generate the active form (6).

Specificity/Sensitivity: Each antibody recognizes endogenous total levels of the specified target protein independent of its modified state.

Source/Purification: Monoclonal antibodies are produced by immunizing animals with a synthetic peptides corresponding to residues surrounding Pro263 of human fructose bisphosphate aldolase A protein, the carboxy terminus of human enolase-2 protein, human PDHK1 protein, residues surrounding Pro454 within the fructose-2,6-biphosphatase region of human PFKFB2 protein, residues surrounding Leu456 of human PFKFB3 protein, and the carboxy terminus of human PGAM1 protein. Polyclonal antibodies are produced by immunizing animals with a synthetic peptide corresponding to the sequence of human enolase-1 and carboxy terminus of human PFKL protein. Polyclonal antibodies are purified by protein A and peptide affinity chromatography.

Background References:

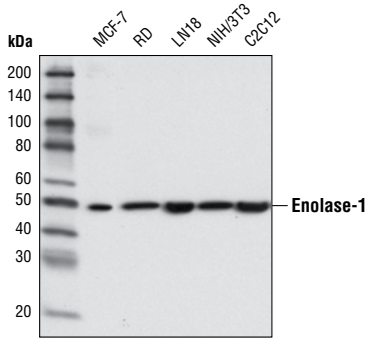
(1) Mediavilla, D. et al. (2008) *J Biochem* 144, 235-44.
(2) Atsumi, T. et al. (2005) *Diabetes* 54, 3349-57.
(3) Castaldo, G. et al. (2000) *Clin Chem* 46, 901-6.
(4) Vander Heiden, M.G. et al. (2010) *Science* 329, 1492-9.
(5) Pancholi, V. (2001) *Cell Mol Life Sci* 58, 902-20.
(6) Wigfield, S.M. et al. (2008) *Br J Cancer* 98, 1975-84.



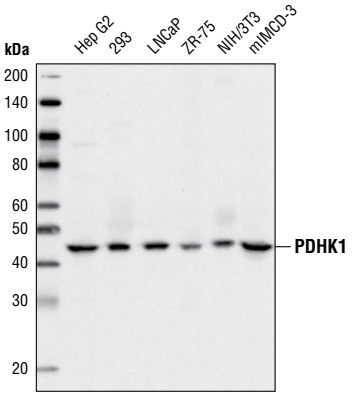
Western blot analysis of extracts from various cell lines using ***Aldolase A (D73H4) Rabbit mAb #8060****.*

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

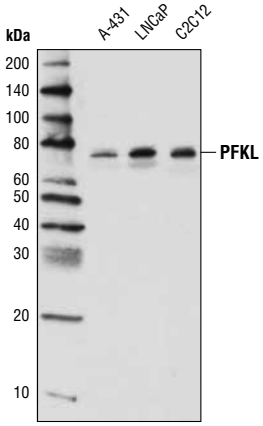
Applications Key: **W**—Western **IP**—Immunoprecipitation **IHC**—Immunohistochemistry **ChIP**—Chromatin Immunoprecipitation **IF**—Immunofluorescence **F**—Flow cytometry **E-P**—ELISA-Peptide
Species Cross-Reactivity Key: **H**—human **M**—mouse **R**—rat **Hm**—hamster **Mk**—monkey **Mi**—mink **C**—chicken **Dm**—D. melanogaster **X**—Xenopus **Z**—zebrafish **B**—bovine **Dg**—dog **Pg**—pig **Sc**—S. cerevisiae **All**—all species expected Species enclosed in parentheses are predicted to react based on 100% homology.



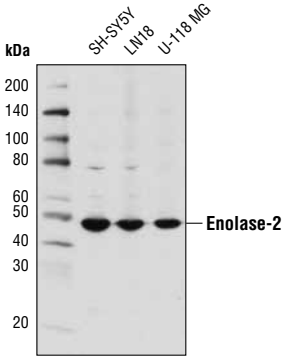
Western blot analysis of extracts from various cell types using ***Enolase-1 Antibody #3810****.*



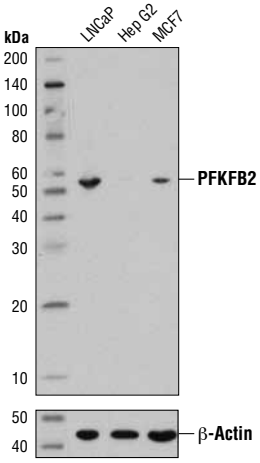
Western blot analysis of extracts from various cell types using ***PDHK1 (C47H1) Rabbit mAb #3820****.*



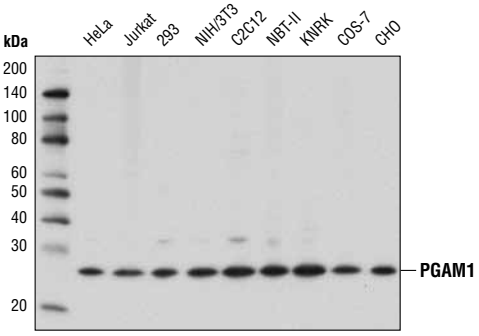
Western blot analysis of extracts from A-431, LNCaP, and C2C12 cells using ***PFKL Antibody #8175****.*



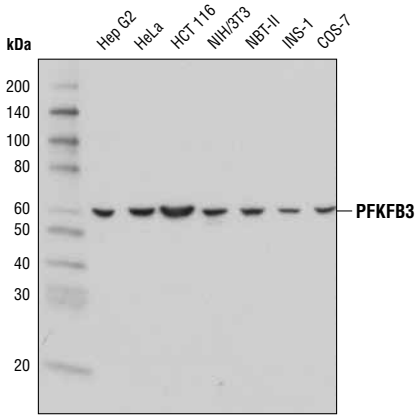
Western blot analysis of extracts from SH-SY5Y, L18, and U-118 MG cells using ***Enolase-2 (D20H2) Rabbit mAb #8171****.*



Western blot analysis of extracts from LNCaP, Hep G2, and MCF7 cells using ***PFKFB2 (D7G5R) Rabbit mAb #13045*** *(upper) and β-Actin (D6A8) Rabbit mAb #8457 (lower).*



Western blot analysis of extracts from various cell lines using ***PGAM1 (D3J9T) Rabbit mAb #12098****.*



Western blot analysis of extracts from various cell lines using ***PFKFB3 (D7H4Q) Rabbit mAb #13123****.*

#12866

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.