Store at -20°C

Tricarboxylic Acid Cycle Antibody Sampler Kit

#47767

1 Kit
(9 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
Fumarase (D9C5) Rabbit mAb	4567	20 µl	49 kDa	Rabbit IgG
IDH2 (D8E3B) Rabbit mAb	56439	20 µl	43 kDa	Rabbit IgG
IDH1 Antibody	3997T	20 µl	46 kDa	Rabbit
Citrate Synthase (D7V8B) Rabbit mAb	14309	20 µl	45 kDa	Rabbit IgG
ACO2 (D6D9) XP® Rabbit mAb	6571	20 µl	85 kDa	Rabbit IgG
MPC2 (D4I7G) Rabbit mAb	46141	20 µl	14 kDa	Rabbit IgG
MPC1 (D2L9I) Rabbit mAb	14462	20 µl	12 kDa	Rabbit IgG
DLST (D22B1) XP® Rabbit mAb	11954	20 µl	50 kDa	Rabbit IgG
SDHA (D6J9M) XP® Rabbit mAb	11998	20 µl	70 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.

Description: The Tricarboxylic Acid Cycle Sampler Kit provides an economical means of detecting select components involved in the tricarboxylic acid cycle. The kit contains enough primary antibodies to perform at least two western blot experiments per antibody.

Background: The tricarboxylic acid cycle (TCA cycle) includes various enzymatic reactions that constitute a key part of aerobic respiration in the cells. The transport of the glycolytic end product pyruvate into mitochondria and the decarboxylation of pyruvate in the TCA cycle generate energy through oxidative phosphorylation under aerobic conditions (1,2). Two inner mitochondrial membrane proteins, mitochondrial pyruvate carrier 1 (MPC1) and mitochondrial pyruvate carrier 2 (MPC2), form a 150 kDa complex and are essential proteins in the facilitated transport of pyruvate into mitochondria (1,2). Citrate synthase catalyzes the first and rate-limiting reaction of the TCA cycle (3). Mitochondrial aconitase 2 (ACO2) catalyzes the conversion of citrate to isocitrate via cis-aconitate (4). IDH1 and IDH2 are two of the three isocitrate dehydrogenases that catalyze oxidative decarboxylation of isocitrate to α-ketoglutarate (α-KG) (5). IDH1 functions as a tumor suppressor in the cytoplasm and peroxisomes whereas IDH2 is in mitochondria and involved in the TCA cycle (5). Mutations in IDH2 have also been identified in malignant gliomas (6). Dihydrolipoamide succinyltransferase (DLST) is a subunit in α-ketoglutarate dehydrogenase complex, a key enzymatic complex in the TCA cycle (7). Succinate dehydrogenase subunit A (SDHA) is a component of the TCA cycle and electron transport chain and is involved in the oxidation of succinate (8). Fumarase catalyzes the conversion of fumarate to malate (9). Fumarase deficiency leads to the accumulation of fumarate, an oncometabolite which causes an epithelial-to-mesenchymal-transition (EMT) of the cells (10).

Specificity/Sensitivity: Each antibody in this kit recognizes endogenous levels of its specific target protein. IDH1 (D2H1) Rabbit mAb does not recognize endogenous IDH2 protein, but does recognize IDH2 when recombinantly overexpressed. IDH2 (D8E3B) Rabbit mAb does not cross-react with IDH1 protein. MPC1 (D2L9I) Rabbit mAb does not cross-react with MPC2 protein. MPC2 (D4I7G) Rabbit mAb does not cross-react with MPC1 protein.

Source/Purification: Monoclonal antibodies are produced by immunizing animals with a synthetic peptide corresponding to residues surrounding Gly540 of human ACO2 protein, residues near the carboxy terminus of human citrate synthase protein, residues surrounding Pro188 of human DLST protein, residues surrounding Gly354 of human fumarase protein, residues surrounding Arg222 of human IDH1 protein, residues surrounding Val195 of human IDH2 protein, residues near the carboxy terminus of human MPC1 protein, residues surrounding Asn33 of human MPC2 protein and residues surrounding Gly166 of human SDHA protein, respectively.

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 validation data and a complete listing of recommended companion products.

Background References:

(1) Herzig, S. et al. (2012) *Science* 337, 93-6.
(2) Bricker, D.K. et al. (2012) *Science* 337, 96-100.
(3) Lin, C.C. et al. (2012) *Sci Rep* 2, 785.
(4) Tohyama, S. et al. (2016) *Cell Metab* 23, 663-74.
(5) Zhao, S. et al. (2009) *Science* 324, 261-5.
(6) Yan, H. et al. (2009) *N Engl J Med* 360, 765-73.
(7) Diaz-Muñoz, M.D. et al. (2015) *Nat Immunol* 16, 415-25.
(8) Renkema, G.H. et al. (2015) *Eur J Hum Genet* 23, 202-9.
(9) Wang, T. et al. (2017) *Nat Cell Biol* 19, 833-843.
(10) Sciacovelli, M. et al. (2016) *Nature* 537, 544-547.

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Applications: **W**—Western **IP**—Immunoprecipitation **IHC**—Immunohistochemistry **ChIP**—Chromatin Immunoprecipitation **IF**—Immunofluorescence **F**—Flow cytometry **E-P**—ELISA-Peptide **Species Cross-Reactivity:** **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.

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.