Store at -20°C

TFEB Signaling Antibody Sampler Kit

#63734

Support: +1-978-867-2388 (U.S.)
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For Research Use Only. Not For Use In Diagnostic Procedures.

Products Included	Product #	Quantity	Mol. Wt.	Isotype/Source
TFEB (D2O7D) Rabbit mAb	37785	20 µl	65-70 kDa	Rabbit IgG
Phospho-TFEB (Ser211) (E9S8N) Rabbit mAb	37681	20 µl	70 kDa	Rabbit IgG
Phospho-TFEB (Ser122) Antibody	86843	20 µl	70-80 kDa	Rabbit
mTOR (7C10) Rabbit mAb	2983	20 µl	289 kDa	Rabbit IgG
Phospho-mTOR (Ser2448) (D9C2) XP® Rabbit mAb	5536	20 µl	289 kDa	Rabbit IgG
Pan-Calcineurin A Antibody	2614	20 µl	59 kDa	Rabbit
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 TFEB Signaling Antibody Sampler Kit provides an economical means of analyzing the regulation of TFEB. The kit includes enough antibodies to perform two western blot experiments with each primary antibody.

Background: Transcription factor EB (TFEB) is a member of the Myc-related, bHLH leucine-zipper family of transcription factors that drives the expression of a network of genes known as the Coordinated Lysosomal Expression and Regulation (CLEAR) network (1,2). TFEB specifically recognizes and binds regulatory sequences within the CLEAR box (GTCACGTGAC) of lysosomal and autophagy genes, resulting in the upregulated expression of genes involved in lysosome biogenesis and function, and regulation of autophagy (1,2). TFEB is activated in response to nutrient deprivation, stimulating translocation to the nucleus where it forms homo- or heterooligomers with other members of the microphthalmia transcription factor (MiTF) subfamily and resulting in upregulation of autophagosomes and lysosomes (3-5). Recently, it has been shown that TFEB is a component of mammalian target of rapamycin (mTOR) complex 1 (mTORC1), which regulates the phosphorylation and nuclear translocation of TFEB in response to cellular starvation and stress (6-9). During normal growth conditions, TFEB is phosphorylated at Ser211 in an mTORC1-dependent manner. Phosphorylation promotes association of TFEB with 14-3-3 family proteins and retention in the cytosol. Inhibition of mTORC1 results in a loss of TFEB phosphorylation, dissociation of the TFEB/14-3-3 complex, and rapid transport of TFEB to the nucleus where it increases transcription of CLEAR and autophagy genes (10). TFEB has also been shown to be activated in a nutrient-dependent manner by p42 MAP kinase (Erk2). TFEB is phosphorylated at Ser142 by Erk2 in response to nutrient deprivation, resulting in nuclear localization and activation, and indicating that pathways other than mTOR contribute to nutrient sensing via TFEB (3). Additional studies have also identified phosphorylation of TFEB at Ser122 that is dependent on mTORC1 (11). mTOR activity is associated with phosphorylation at Ser2448 via the PI3 kinase/Akt signaling pathway (12). Lysosomal calcium release activates the phosphatase calcineurin that dephosphorylates TFEB and promotes nuclear translocation and autophagy (13).

Specificity/Sensitivity: Each antibody in the TFEB Signaling Antibody Sampler Kit detects endogenous levels of its target protein. Pan-Calcineurin A Antibody detects endogenous levels of the alpha isoform of Calcineurin A. It may also recognize the beta and gamma isoforms of Calcineurin A. The antibody does not cross-react with protein phosphatase 1 or 2A.

Source/Purification: Monoclonal antibodies are produced by immunizing animals with synthetic peptides corresponding to residues surrounding Gly412 of human TFEB and Ser2481 of human mTOR, and synthetic phosphopeptides corresponding to residues surrounding Ser211 of human TFEB and Ser2448 of human mTOR. Polyclonal antibodies are produced by immunizing animals with a synthetic phosphopeptide surrounding Ser122 and a synthetic peptide corresponding to the carboxy terminus of human Calcineurin A (alpha isoform). Antibodies are purified by protein A and peptide affinity chromatography.

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.*

Please visit www.cellsignal.com for validation data and a complete listing of recommended companion products.

Background References:

(1) Sardiello, M. et al. (2009) *Science* 325, 473-7.
(2) Sardiello, M. and Ballabio, A. (2009) *Cell Cycle* 8, 4021-2.
(3) Settembre, C. et al. (2011) *Science* 332, 1429-33.
(4) David, R. (2011) *Nat Rev Mol Cell Biol* 12, 404.
(5) Cuervo, A.M. (2011) *Science* 332, 1392-3.
(6) Peña-Llopis, S. et al. (2011) *EMBO J* 30, 3242-58.
(7) Settembre, C. and Ballabio, A. (2011) *Autophagy* 7, 1379-81.
(8) Peña-Llopis, S. and Brugarolas, J. (2011) *Cell Cycle* 10, 3987-8.
(9) Settembre, C. et al. (2012) *EMBO J* 31, 1095-108.
(10) Martina, J.A. et al. (2012) *Autophagy* 8, 903-14.
(11) Vega-Rubin-de-Celis, S. et al. (2017) *Autophagy* 13, 464-472.
(12) Navé, B.T. et al. (1999) *Biochem J* 344 Pt 2, 427-31.
(13) Medina, D.L. et al. (2015) *Nat Cell Biol* 17, 288-99.

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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.