Store at –20°C

#4763

Translation Initiation Complex Antibody Sampler Kit

☑ 1 Kit
(9 x 20 µl)

Cell Signaling TECHNOLOGY®

Orders ■ 877-616-CELL (2355)
orders@cellsignal.com
Support ■ 877-678-TECH (8324)
info@cellsignal.com
Web ■ www.cellsignal.com

rev. 06/16

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

Products Included	Product #	Quantity	Mol. Wt.	Isotype
eIF4A (C32B4) Rabbit mAb	2013	20 µl	48 kDa	Rabbit IgG
eIF4A1 Antibody	2490	20 µl	48 kDa	Rabbit IgG
Phospho-eIF4B (Ser422) Antibody	3591	20 µl	80 kDa	Rabbit IgG
eIF4B Antibody	3592	20 µl	80 kDa	Rabbit IgG
Phospho-eIF4E (Ser209) Antibody	9741	20 µl	25 kDa	Rabbit IgG
eIF4E (C46H6) Rabbit mAb	2067	20 µl	25 kDa	Rabbit IgG
Phospho-eIF4G (Ser1108) Antibody	2441	20 µl	220 kDa	Rabbit IgG
eIF4G (C45A4) Rabbit mAb	2469	20 µl	220 kDa	Rabbit IgG
eIF4H (D85F2) XP® Rabbit mAb	3469	20 µl	25, 27 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 and 50% glycerol. 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.

Description: The Translation Initiation Complex Antibody Sampler Kit contains reagents to investigate the initiation of translation within the cell. The kit includes enough antibody to perform two Western blot experiments with each primary antibody.

Background: A variety of factors contribute to the important biological event of translation initiation. The Eukaryotic initiation Factor 4E (eIF4E) complex of translation initiation factors binds to the 5' m7 GTP cap to open up the mRNA secondary structure and allow small ribosome subunit binding (1). eIF4A, an eIF4 complex component that acts as an ATP-dependent RNA helicase, unwinds the secondary structure of the 5' mRNA untranslated region to mediate ribosome binding (2,3). EIF4E binds to the mRNA cap structure to mediate the initiation of translation (4,5). eIF4E interacts with eIF4G, a scaffold protein that promotes assembly of eIF4E and eIF4A into the eIF4F complex (5). eIF4B is thought to assist the eIF4F complex in translation initiation. eIF4H induces the RNA-dependent ATP hydrolysis catalyzed by the initiation factors eIF4A and eIF4B (2,6). eIF4H was further shown to determine the initial rate and extent of eIF4A-mediated mRNA secondary structure unwinding (7).

Specificity/Sensitivity: Each antibody in the Translation Initiation Complex Antibody Sampler Kit detects endogenous levels of its target protein.

Source/Purification: Monoclonal antibodies are produced by immunizing animals with a synthetic petide corresponding to residues surrounding Met316 of human eIF4A protein, Gly188 of human eIF4G, and the sequence of human eIF4E and human eIF4H. Polyclonal antibodies are produced by immunizing animals with a synthetic peptide corresponding to a sequence around Gly12 of human eIF4A1, residues at the amino terminus of human eIF4B, Ser422 of human eIF4B, Ser209 of human eIF4E, and Ser1108 of human eIF4GI. Polyclonal antibodies are purified by protein A and peptide affinity chromatography.

Background References:

(1) Rogers, G.W. et al. (2001) *J Biol Chem* 276, 12598–608.
(2) Rogers, G.W. et al. (1999) *J Biol Chem* 274, 12236–44.
(3) Svitkin, Y.V. et al. (2001) *RNA* 7, 382–94.
(4) Sonenberg, N. et al. (1978) *Proc Natl Acad Sci USA* 75, 4843–7.
(5) Gingras, A.C. et al. (1999) *Annu Rev Biochem* 68, 913–63.
(6) Richter-Cook, N.J. et al. (1998) *J Biol Chem* 273, 7579–87.
(7) Rogers, G.W. et al. (2001) *J Biol Chem* 276, 30914–22.

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 **Ce**—C. elegans **Hr**—Horse **All**—all species expected Species enclosed in parentheses are predicted to react based on 100% homology.

#4763

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.