

#2068 Store at -20°C

# eIF3C Antibody



✓ 100 µl  
(10 Western mini-blot)

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

New 05/08

This product is for *in vitro* research use only and is not intended for use in humans or animals.  
This product is not intended for use as a therapeutic or in diagnostic procedures.

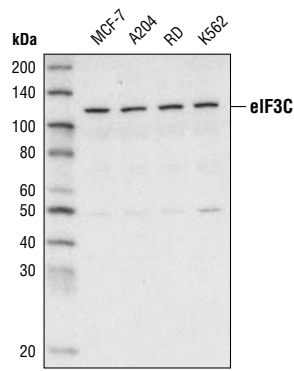
Applications	Species Cross-Reactivity*	Molecular Wt.	Source
W Endogenous	H, M, Mk, (R)	110 kDa	Rabbit

**Background:** Translation initiation requires a set of factors to facilitate the association of the 40S ribosomal subunit with mRNA. The eIF4F complex, consisting of eIF4E, eIF4A and eIF4G, binds to the 5' cap structure of mRNA. eIF4F and eIF4B unwind the secondary structure of mRNA at its 5' untranslated region. The 40S ribosomal subunit, along with some initiation factors including eIF3, then binds to the 5' mRNA cap and searches along the mRNA for the initiation codon. eIF3 is a large translation initiation complex with 10 to 13 different subunits. eIF3a, eIF3b, eIF3c, eIF3e, eIF3f and eIF3h are the core subunits critical for the function of this complex. eIF3 physically interacts with eIF4G, which may be responsible for the association of the 40S ribosomal subunit with mRNA (1). eIF3 also stabilizes the binding of Met-tRNA<sup>f</sup>.eIF2.GTP to the 40S ribosomal subunit and helps keep the integrity of the resulting complex upon addition of the 60S ribosomal subunit (2). Studies have shown that mTOR interacts with eIF3 directly (3,4). When cells are stimulated by hormones or mitogenic signals, mTOR binds to the eIF3 complex and phosphorylates S6K1 (3). This process results in the dissociation of S6K1 from eIF3 and S6K1 activation. The activated S6K1 then phosphorylates its downstream targets including ribosomal protein S6 and eIF4B resulting in stimulation of translation. Further findings demonstrated that activated mTOR signaling induces the association of eIF3 with eIF4G upon stimulation with insulin (3).

**Specificity/Sensitivity:** eIF3C Antibody detects endogenous levels of total eIF3C protein.

**Source/Purification:** Polyclonal antibodies are produced by immunizing animals with a synthetic peptide (KLH-coupled) derived from a sequence of human eIF3C. Antibodies are purified by protein A and peptide affinity chromatography.

- Background References:**
- (1) Masutani, M. et al. (2007) *EMBO J* 26, 3373–83.
  - (2) Chaudhuri, J. et al. (1999) *J Biol Chem* 274, 17975–80.
  - (3) Holz, M.K. et al. (2005) *Cell* 123, 569–80.
  - (4) Harris, T.E. et al. (2006) *EMBO J* 25, 1659–68.



Western blot analysis of extracts from various cell lines using eIF3C Antibody.

Entrez-Gene ID #8663  
Swiss-Prot Acc. #Q99613

**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 antibody.

\*Species cross-reactivity is determined by Western blot.

**Recommended Antibody Dilutions:**  
Western blotting 1:1000

- Companion Products:**
- eIF4G1 Antibody #2858
  - Phospho-eIF4G (Ser1108) Antibody #2441
  - eIF4G (C45A4) Rabbit mAb #2469
  - eIF4G (C65H5) Rabbit mAb #2617
  - eIF4G Antibody #2498
  - eIF4A (F52) Antibody #2425
  - eIF4A (C32B4) Rabbit mAb #2013
  - eIF4A1 Antibody #2490
  - Phospho-eIF4E (Ser209) Antibody #9741
  - eIF4E Antibody #9742
  - 4E-BP1 (53H11) Rabbit mAb #9644
  - eIF4H Antibody #2444
  - eIF5 Antibody #2480
  - PABP1 Antibody #4992
  - Ribosomal Protein L13a Antibody #2765
  - Tris Buffered Saline with Tween 20 (TBST - 10X) #9997
  - BSA #9998
  - Nonfat Dry Milk #9999
  - Phototope<sup>®</sup>-HRP Western Blot Detection System, Anti-rabbit IgG, HRP-linked Antibody #7071
  - Anti-rabbit IgG, HRP-linked Antibody #7074
  - Prestained Protein Marker, Broad Range (Premixed Format) #7720
  - Biotinylated Protein Ladder Detection Pack #7727
  - 20X LumiGLO<sup>®</sup> Reagent and 20X Peroxide #7003

**IMPORTANT: 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.**

**Applications Key:** W—Western IP—Immunoprecipitation IHC—Immunohistochemistry ChIP—Chromatin Immunoprecipitation IF—Immunofluorescence F—Flow cytometry E—ELISA  
**Species Cross-Reactivity Key:** H—human M—mouse R—rat Hm—hamster Mk—monkey Mi—mink C—chicken Dm—D. melanogaster X—Xenopus Z—zebra fish 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.

## 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<sup>®</sup>-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<sup>®</sup> 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<sup>2</sup>) 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<sup>®</sup> (0.5 ml 20X LumiGLO<sup>®</sup>, 0.5 ml 20X Peroxide and 9.0 ml Milli-Q water) with gentle agitation for 1 minute at room temperature.

**NOTE:** LumiGLO<sup>®</sup> 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<sup>®</sup> incubation and declines over the following 2 hours.