

# Pro-TGF- $\alpha$ Antibody

✓ 100  $\mu$ l  
(10 Western mini-blot)



## Cell Signaling TECHNOLOGY®

Orders ■ 877-616-CELL (2355)  
orders@cellsignal.com

Support ■ 877-678-TECH (8324)  
info@cellsignal.com

Web ■ www.cellsignal.com

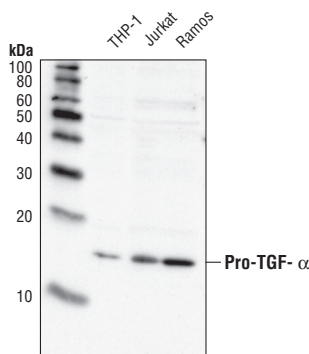
new 07/04

Applications W	Species Cross-Reactivity H, M (R)	Molecular Wt. 17 kDa	Source Rabbit
-------------------	--------------------------------------	-------------------------	------------------

**Background:** Transforming growth factor  $\alpha$  (TGF- $\alpha$ ) is a member of the epidermal growth factor (EGF) family, sharing the same receptor, EGFR, and regulating cell proliferation, survival, and differentiation (1). Members of the family share an EGF-like domain of 45-60 amino acids characterized by the conservation of six regularly spaced cysteines, forming three disulfide bonds that function as their receptor binding domain. TGF- $\alpha$  was initially discovered in the media of retrovirally transformed fibroblasts, and its name comes from its ability to induce transformation in cultured fibroblasts (2). This transforming activity was later shown to require TGF- $\beta$ , which potentiates the activity of TGF- $\alpha$  through a separate receptor (3). Soluble TGF- $\alpha$  is released from its membrane-bound precursor, pro-TGF- $\alpha$ , following proteolytic cleavage, but the membrane bound precursor is still able to bind and activate EGFR (4). Binding of soluble or membrane bound TGF- $\alpha$  to EGFR leads to receptor dimerization, tyrosine autophosphorylation, and activation of downstream signaling components. TGF- $\alpha$  and related peptides play an important role in the progression of cancer as well as in neuropathological processes (5,6).

**Specificity/Sensitivity:** Pro-TGF- $\alpha$  Antibody detects endogenous levels of TGF- $\alpha$  precursor protein but not mature TGF- $\alpha$ . It does not cross-react with other family members at physiological levels.

**Source/Purification:** Polyclonal antibodies are produced by immunizing rabbits with a synthetic peptide (KLH-coupled) corresponding to the amino-terminal sequence of TGF- $\alpha$ . Antibodies are purified by protein A and peptide affinity chromatography.



Western blot analysis of extracts from THP-1, Jurkat, and Ramos cells, using Pro-TGF- $\alpha$  Antibody.

### Background References:

- (1) Derynck, R. (1986) *J. Cell Biochem.* 32, 203-204.
- (2) de Larco, J.E. and Tordaro, G.J. (1978) *Proc. Natl. Acad. Sci. USA* 75, 4001-4005.
- (3) Roberts, A.B. et al. (1981) *Proc. Natl. Acad. Sci. USA* 78, 5339-5343.
- (4) Wong, S.T. et al. (1989) *Cell* 56, 495-506.
- (5) Rusch, V. et al. (1996) *Cytokine Growth Factor Rev.* 7, 133-141.
- (6) Junier, M.P. (2000) *Prog. Neurobiol.* 62, 443-473.

**Storage:** Supplied in 10 mM sodium HEPES (pH 7.5), 150 mM NaCl, 100  $\mu$ g/ml BSA and 50% glycerol. Store at -20°C. Do not aliquot the antibody.

### Recommended Antibody Dilutions:

Western blotting 1:1000

### Companion Products:

Phototope®-HRP Western 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

LumiGLO® Reagent and Peroxide #7003

**IMPORTANT: For Western blots, incubate membrane with diluted antibody in 5% BSA, 1X TBS, 0.1% Tween-20 at 4°C with gentle shaking, overnight.**

Applications Key: W—Western IP—Immunoprecipitation IHC—Immunohistochemistry IC—Immunocytochemistry F—Flow cytometry E—ELISA D—DELFIATM  
Species Cross-Reactivity Key: H—human M—mouse R—rat Hm—hamster Mk—monkey Mi—mink C—chicken X—Xenopus Z—zebra fish All—all species expected  
Species enclosed in parentheses are predicted to react based on 100% sequence homology.

## Western Immunoblotting Protocol

**For Western blots, incubate membrane with diluted anti-body in 5% 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.

- A1. ■ 1X Phosphate Buffered Saline (PBS)
- A2. ■ 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
- A3. ■ Transfer Buffer:  
25 mM Tris base, 0.2 M glycine, 20% methanol (pH 8.5)
- A4. ■ 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).
- A5. ■ Nonfat Dry Milk (weight to volume [w/v])
- A6. ■ 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%).
- A7. ■ Wash Buffer:  
1X TBS, 0.1% Tween-20 (TBS/T)
- A8. ■ Bovine Serum Albumin (BSA)
- A9. ■ 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%).
- A10. ■ 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.
- A11. ■ Prestained Protein Marker, Broad Range (Premixed Format) #7720
- A12. ■ Biotinylated Protein Ladder Detection Pack #7727
- A13. ■ 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.

- B1. Treat cells by adding fresh media containing regulator for desired time.
- B2. Aspirate media from cultures; wash cells with 1X PBS; aspirate.
- B3. Lyse cells by adding 1X SDS sample buffer (100 µl per well of 6-well plate or 500 µl per plate of 10 cm plate). Immediately scrape the cells off the plate and transfer the extract to a microcentrifuge tube. Keep on ice.
- B4. Sonicate for 10–15 seconds to shear DNA and reduce sample viscosity.
- B5. Heat a 20 µl sample to 95–100°C for 5 minutes; cool on ice.
- B6. Microcentrifuge for 5 minutes.
- B7. 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.*

- B8. 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.*

- C1. (Optional) After transfer, wash nitrocellulose membrane with 25 ml TBS for 5 minutes at room temperature.
- C2. Incubate membrane in 25 ml of blocking buffer for 1 hour at room temperature.
- C3. Wash three times for 5 minutes each with 15 ml of TBS/T.
- C4. Incubate membrane and primary antibody (at the appropriate dilution) in 10 ml primary antibody dilution buffer with gentle agitation overnight at 4°C.
- C5. Wash three times for 5 minutes each with 15 ml of TBS/T.
- C6. 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.
- C7. Wash three times for 5 minutes each with 15 ml of TBS/T.

**D. Detection of Proteins**

- D1. 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.*

- D2. 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.*