

# TGF-β Receptor III Antibody

100 μl  
 (10 Western mini-blot)

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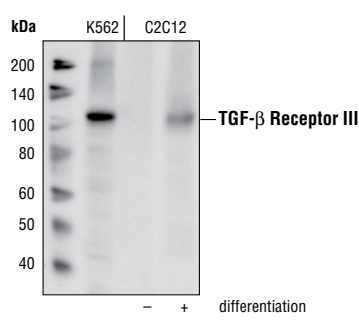
This product is for *in vitro* research use only and is not intended for use in humans or animals.

Applications W, IP	Species Cross-Reactivity* H, M, (R)	Molecular Wt. 110 kDa	Source Rabbit
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**Background:** Transforming growth factor-β (TGF-β) superfamily members are critical regulators of cell proliferation and differentiation, developmental patterning and morphogenesis, and disease pathogenesis (1-4). TGF-β elicits signaling through three cell surface receptors: type I (RI), type II (RII) and type III (RIII). Type I and type II receptors are serine/threonine kinases that form a heteromeric complex. In response to ligand binding, the type II receptors form a stable complex with the type I receptors allowing phosphorylation and thus activation of the type I receptor kinases (5). The type III receptor, also known as betaglycan, is a transmembrane proteoglycan with a large extracellular domain that binds TGF-β with high affinity but lacks a cytoplasmic signaling domain (6,7). Expression of the type III receptor can regulate TGF-β signaling through presentation of the ligand to the signaling complex. The only known direct TGF-β signaling effectors are the Smads, which function by transducing signals from the cell surface directly to the nucleus and regulating transcription of target genes (8,9). TGF-β type III receptor is upregulated during skeletal muscle differentiation (10).

**Specificity/Sensitivity:** TGF-β Receptor III Antibody detects endogenous levels of the receptor. No cross reactivity was detected with other family members at physiological conditions.

**Source/Purification:** Polyclonal antibodies are produced by immunizing rabbits with a synthetic peptide (KLH-coupled) corresponding to residues in the extracellular domain of the type III TGF-β receptor. Antibodies are purified by protein A and peptide affinity chromatography.



Western blot analysis of extracts from K562 and C2C12 cells upon differentiation into myoblasts using TGF-β Receptor III Antibody.

**Background References:**

- (1) Massague, J. et al. (2000) *Cell* 103, 295–309.
- (2) Caestecker, M.P. et al. (2000) *J. Natl. Cancer Inst.* 92, 1388–1402.
- (3) Derynck, R. et al. (2001) *Nature Genet.* 29, 117–129.
- (4) Miyazono, K. et al. (2000) *Adv. Immunol.* 75, 115–157.
- (5) Derynck, R. et al. (1997) *Biochim. Biophys. Acta.* 1333, F105–150.
- (6) Lopez-Casillas, F. et al. (1991) *Cell* 67, 785–795.
- (7) Wang, X.F. et al. (1991) *Cell* 67, 797–805.
- (8) Derynck, R. et al. (1998) *Cell* 95, 737–740.
- (9) Massague, J. et al. (2000) *Nat. Rev. Mol. Cell Biol.* 1, 169–178.
- (10) Lopez-Casillas, F. et al. (2003) *J. Biol. Chem.* 278, 382–390.

**Entrez-Gene ID** #7049  
**Swiss-Prot Acc.** #Q03167

**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  
 Immunoprecipitation 1:100

**Companion Products:**

- TGF-β Receptor I Antibody #3712
- TGF-β (56E4) Rabbit mAb #3709
- TGF-β Antibody #3711
- Smad2/3 Antibody #3102
- Phospho-Smad2 (Ser465/467) Antibody #3101
- Phospho-Smad3 (Ser423/425) (C25A9) Rabbit mAb #9520
- Phototope®-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 #7727
- 20X LumiGLO® 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.**

## 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.

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

- Treat cells by adding fresh media containing regulator for desired time.
- Aspirate media from cultures; wash cells with 1X PBS; aspirate.
- 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.
- Sonicate for 10–15 seconds to shear DNA and reduce sample viscosity.
- Heat a 20 µl sample to 95–100°C for 5 minutes; cool on ice.
- Microcentrifuge for 5 minutes.
- 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.

- 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.

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

### D Detection of Proteins

- 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.

- 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.

## Immunoprecipitation Protocol / (For Analysis By Western Immunoblotting)

### A Solutions and Reagents

**NOTE:** Prepare solutions with Milli-Q or equivalently purified water.

- 1X Phosphate Buffered Saline (PBS)
- 1X Cell Lysis Buffer:** 20 mM Tris (pH 7.5), 150 mM NaCl, 1 mM EDTA, 1 mM EGTA, 1% Triton X-100, 2.5 mM Sodium pyrophosphate, 1 mM  $\beta$ -glycerophosphate, 1 mM  $\text{Na}_3\text{VO}_4$ , 1  $\mu\text{g/ml}$  Leupeptin

**NOTE:** Add 1 mM PMSF immediately prior to use.

- Transfer Buffer:** 25 mM Tris base, 0.2 mM glycine, 20% methanol (pH 8.5)
- Protein A or G Agarose Beads:** (Can be stored for 2 weeks at 4°C.) Please prepare according to manufacturer's instructions. Use Protein A for rabbit IgG pull down and Protein G for mouse IgG pull down.
- 3X SDS Sample Buffer:** 187.5 mM Tris-HCl (pH 6.8 at 25°C), 6% w/v SDS, 30% glycerol, 150 mM DTT, 0.03% w/v bromophenol blue

### B Preparing Cell Lysates

- Aspirate media. Treat cells by adding fresh media containing regulator for desired time.
- To harvest cells under nondenaturing conditions, remove media and rinse cells once with ice-cold PBS.
- Remove PBS and add 0.5 ml ice-cold 1X cell lysis buffer to each plate (10 cm) and incubate the plates on ice for 5 minutes.
- Scrape cells off the plates and transfer to microcentrifuge tubes. Keep on ice.
- Sonicate samples on ice three times for 5 seconds each.
- Microcentrifuge for 10 minutes at 14,000 X g, 4°C, and transfer the supernatant to a new tube. If necessary, lysate can be stored at -80°C.

### C Immunoprecipitation

Optional: It may be necessary to perform a lysate pre-clearing step to reduce non-specific binding to the Protein A/G agarose beads (See section below).

- Take 200  $\mu\text{l}$  cell lysate and add primary antibody. Incubate with gentle rocking overnight at 4°C.
- Add either protein A or G agarose beads (20  $\mu\text{l}$  of 50% bead slurry). Incubate with gentle rocking for 1–3 hours at 4°C.
- Microcentrifuge for 30 seconds at 4°C. Wash pellet five times with 500  $\mu\text{l}$  of 1X cell lysis buffer. Keep on ice during washes.
- Resuspend the pellet with 20  $\mu\text{l}$  3X SDS sample buffer. Vortex, then microcentrifuge for 30 seconds.
- Heat the sample to 95–100°C for 2–5 minutes and microcentrifuge for 1 minute at 14,000 X g.
- Load the sample (15–30  $\mu\text{l}$ ) on SDS-PAGE gel (12–15%).
- Analyze sample by Western blotting (see Western Immunoblotting Protocol).

### Cell Lysate Pre-Clearing (Optional)

- Take 200  $\mu\text{l}$  cell lysate and add to either Protein A or G agarose beads (20  $\mu\text{l}$  of 50% bead slurry).
- Incubate at 4°C for 30 – 60 minutes.
- Spin for 10 minutes at 4°C. Transfer the supernatant to a fresh tube.
- Proceed to step 1 of Immunoprecipitation.