

#4045 Store at -20°C

WWOX Antibody



100 µl
 (10 Western mini-blot)

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New 06/07

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

| Applications | Species Cross-Reactivity* | Molecular Wt. | Source |
|--------------|---------------------------|---------------|--------|
| W | H, M, R, (Mk) | 46 kDa | Rabbit |

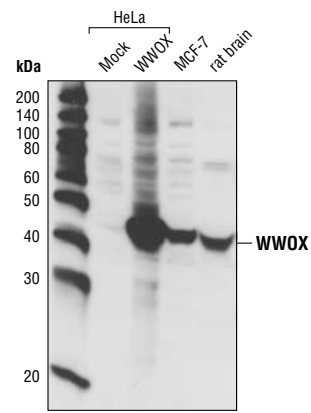
Background: The *WWOX* (WW domain-containing oxidoreductase) gene encodes a protein with two WW domains followed by a short-chain dehydrogenase domain that was identified from a genomic region 16q23 of high instability, FRA16D (1,2). The mouse homolog, termed *Wox1*, was found to enhance TNF α -mediated apoptosis (3). The *WWOX* gene is disrupted in a many cancer types by deletions or translocation which has revealed a tumor suppressor function (4-7). In contrast, high levels of *WWOX* have been shown in shown in premetastatic cancers, including breast and prostate (8-10). Stress stimuli can induce tyrosine phosphorylation within the first WW domain (Tyr33), followed by nuclear translocation and binding to and stabilizing the p53 tumor suppressor protein (11). *WWOX* and p53 can induce apoptosis in a synergistic manner. Tyrosine phosphorylation and nuclear translocation of *WWOX* has been implicated in the progression of cancers to metastatic states (10).

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

Source/Purification: Polyclonal antibodies were prepared by immunizing rabbits with a synthetic peptide (KLH-coupled) corresponding to residues surrounding Thr103 of *WWOX*. Antibodies were purified by peptide affinity chromatography.

Background References:

- (1) Bednarek, A.K. et al. (2000) *Cancer Res.* 60, 2140–2145.
- (2) Ried, K. et al. (2000) *Hum. Mol. Genet.* 9, 1651–1663.
- (3) Chang, N.S. et al. (2001) *J. Biol. Chem.* 276, 3361–3370.
- (4) Ramos, D. and Aldaz, C.M. (2006) *Adv. Exp. Med. Biol.* 587, 149–159.
- (5) Paige, A.J. et al. (2001) *Proc. Natl. Acad. Sci. USA* 98, 11417–11422.
- (6) Bednarek, A.K. et al. (2001) *Cancer Res.* 61, 8068–8073.
- (7) Aqeilan, R.I. et al. (2007) *Proc. Natl. Acad. Sci. USA* 104, 3949–3954.
- (8) Driouch, K. et al. (2002) *Oncogene* 21, 1832–1840.
- (9) Watanabe, A. et al. (2003) *Cancer Res.* 63, 8629–8633.
- (10) Chang, N.S. et al. (2005) *Oncogene* 24, 714–723.
- (11) Chang, N.S. et al. (2005) *J. Biol. Chem.* 280, 43100–43108.



Western blot analysis of extracts from HeLa cells, mock transfected or transfected with mouse *WWOX*, and from MCF-7 and rat brain using *WWOX* Antibody.

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:**
- Phospho-p53 (Ser46) Antibody #2521
 - p53 (7F5) Rabbit mAb #2527
 - p53 (1C12) Mouse mAb #2524
 - 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 Detection Pack #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.

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

Western Immunoblotting Protocol (Primary Ab 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 cm²) 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.