

#4892 Store at -20°C

p63-α Antibody

✓ 100 µl
(10 Western mini-blot)

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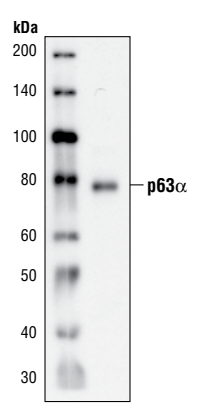
Applications	Species Cross-Reactivity*	Molecular Wt.	Source
W, IF-IC Endogenous	H, (C)	75 kDa	Rabbit

Background: The p53 tumor suppressor protein plays a major role in cellular response to DNA damage and other genomic aberrations. Activation of p53 can lead to either cell cycle arrest and DNA repair or apoptosis (1). In addition to p53, mammalian cells contain two p53 family members, p63 and p73, which are similar to p53 in both structure and function (2). While p63 can induce p53-responsive genes and apoptosis, mutation of p63 rarely results in tumors (2). Amplification of the p63 gene is frequently observed in squamous cell carcinomas of the lung, head and neck (2,3). The p63 gene contains an alternative transcription initiation site that yields a 40 kDa δNp63 lacking the transactivation domain, and alternative splicing at the carboxy-terminus yields the α, β and γ isoforms (3,4).

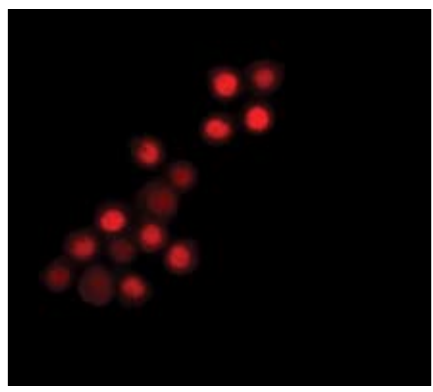
Specificity/Sensitivity: p63alpha Antibody detects endogenous levels of total p63α protein. This antibody will detect both full length TAp63α and truncated δNp63α, but not p63β or p63γ.

Source/Purification: Polyclonal antibodies are produced by immunizing rabbits with a synthetic peptide (KLH-coupled) corresponding to residues within the carboxy-terminus of p63α. Antibodies are purified by protein A and peptide affinity chromatography.

- Background References:**
- (1) Levine, A.J. et al. (1997) *Cell* 88, 323–331.
 - (2) Waltermann, A. et al. (2003) *Oncogene* 22, 5686–5693.
 - (3) Hibi, K. et al. (2000) *Proc. Natl. Acad. Sci. USA* 97, 5462–5467.
 - (4) Yang, A. et al. (1999) *Nature* 398, 714–718.



Western blot analysis of extracts from ME-180 cells, using p63α Antibody.



Immunofluorescent analysis of ME-180 cells, using p63α Antibody.

Entrez-Gene ID #8626
Swiss-Prot Acc. #Q9H3D4

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
Immunofluorescence (IF-IC) 1:100

- Companion Products:**
- p53 (1C12) Mouse mAb #2524
 - p73 Antibody #4662
 - SignalSilence® p73 siRNA Kit #6370
 - SignalSilence® p73 siRNA #6371
 - 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.

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 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.

Immunocytochemistry Protocol for Immunofluorescence

This procedure works well when cells are grown in 6-well tissue culture plates containing sterile coverslips in the appropriate media and concentration of fetal bovine serum (FBS). If desired, provide extra coverslips for cell staining controls.

A Solutions and Reagents

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

1. Fetal Bovine Serum (FBS)
2. **10X Phosphate Buffered Saline (PBS):** To prepare 1L add 80 g sodium chloride (NaCl), 2 g potassium chloride (KCl), 14.4 g sodium phosphate, dibasic (Na_2HPO_4) and 2.4 g potassium phosphate, monobasic (KH_2PO_4) to 1 L dH_2O . Adjust pH to 7.4.
3. **4% Paraformaldehyde:** Prepare day of use either from commercial 16% stock solution or by dissolving paraformaldehyde in 1X PBS by heating at 60°C and stirring until it dissolves. Cool before use.
4. **Tris Buffered Saline (TBS):** 50 mM Tris-HCl (pH 7.4), 150 mM NaCl.
5. **0.1% Sodium Borohydride:** Dissolve in PBS on day of use.
6. **0.2% Triton X-100:** Prepare stock of 20% Triton in PBS; rotate tube overnight to dissolve. Dilute to 0.2%.
7. 1% Bovine Serum Albumin (BSA)
8. **Blocking Buffer:** 10% horse or goat serum, 1% BSA, 0.02% NaN₃, 1X PBS.
9. **ProLong™ Antifade Kit:** As directed by Molecular Probes (#P-7481), prepare just before use. Add approximately 1 ml of ProLong mounting medium to one vial of ProLong antifade reagent. Mix gently. Any unused mixture can be stored at -20°C (to slow reaction) for up to one month.

B Fixation

1. Prepare 4% paraformaldehyde and/or cool 100% methanol to -20°C in a tightly sealed container.
2. Treat cells as desired.
3. Wash cells on coverslips once with cool or room temperature TBS.
4. Aspirate off TBS completely and immediately fix as appropriate for your samples.

C Methanol (protein precipitation) Fixation

1. Immerse coverslips in -20°C 100% methanol for 10 minutes.
2. Rinse slips three times for 5 minutes each with room temperature TBS.
3. Quench cells in fresh 0.1% sodium borohydride in TBS for 5 minutes.
4. Wash slips three times for 5 minutes each with room temperature TBS. Aspirate off completely and return slips to TBS only. Continue protocol at "Blocking" step E1.

D Paraformaldehyde (cross-linking) Fixation

1. Immerse coverslips in 4% paraformaldehyde at room temperature for 10 minutes.
2. Wash coverslips once with TBS. Aspirate completely and then permeabilize cells on coverslips with 0.2% Triton X-100 for 5 minutes at room temperature or alternatively with -20°C methanol for 5 minutes.
3. Wash slips three times for 5 minutes each with TBS at room temperature.
4. Quench cells in fresh 0.1% sodium borohydride in TBS for 5 minutes. Aspirate off completely and return slips to TBS only. Continue protocol at blocking step.

E Blocking

1. Block all slips with blocking buffer at room temperature for 45–60 minutes. Wash once for 5 minutes with TBS.

F Staining

1. Dilute the primary antibody as appropriate in 1% BSA in TBS. Centrifuging the antibody for 20 minutes at 12,000 x g in a refrigerated microcentrifuge prior to use will remove any aggregated material, thereby reducing background. Apply the diluted antibody to the cells on coverslips and, most critically, incubate overnight at 4°C.

NOTE: When using any primary or fluorescence-labeled secondary antibody for the first time, titrate out the antibody to determine which dilution allows for the strongest specific signal with the least background for your sample.

NOTE: You may wish to leave one slip for a secondary antibody-only control.

2. Wash all slips three times for 5 minutes each with TBS.
3. Incubate all slips with a dilution of the fluorescence-labeled secondary antibody in 1% BSA in TBS for 30–45 minutes at room temperature in the dark.
4. Wash all slips three times for 5 minutes each with TBS in low lighting conditions.
5. Mount coverslips on slides using the ProLong™ Antifade Kit. Store slides at room temperature in the dark.