

#3742 Store at -20°C

E2F-1 Antibody

✓ 100 µl
(10 Western mini-blot)



Cell Signaling TECHNOLOGY®

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Applications W	Species Cross-Reactivity H, M	Molecular Wt. 70 kDa	Source Rabbit
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Background: The E2F transcription factors are essential for regulation of the cell cycle (1,2). Physiological E2F is a heterodimer composed of an E2F subunit together with a DP subunit (3, 4). Six members of the E2F family have been identified, and each E2F subunit has a DNA binding and a dimerization domain. E2F-1 to -5 activate transcription. E2F-1 to -3 bind pRb, and E2F-4 and -5 bind p107 or p130, and these interactions are under cell cycle control (5-8). E2F-1 has oncogenic properties *in vivo* and *in vitro*. E2F-1 can induce apoptosis through p53-dependent and -independent mechanisms. E2F-1 is stress-responsive, and is regulated by a PI3-kinase-like kinase family such as the ATM/ATR kinases (9-11).

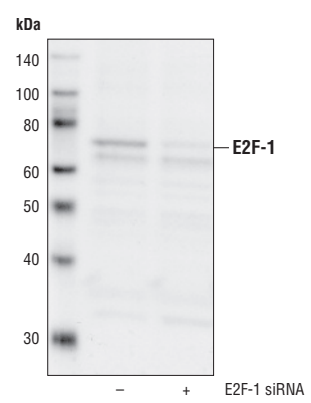
Specificity/Sensitivity: E2F-1 Antibody detects endogenous levels of total E2F-1 protein. The antibody does not cross-react with other proteins.

Source/Purification: Polyclonal antibodies are produced by immunizing rabbits with a synthetic peptide (KLH-coupled) corresponding to the carboxy-terminal residues of human E2F-1. Antibodies are purified by protein A and peptide affinity chromatography.

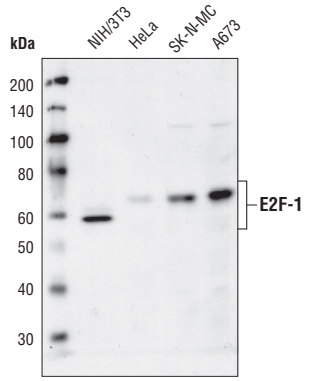
Background References:

- (1) Helin, K. (1998) *Curr. Opin. Genet. Dev.* 8, 28–35.
- (2) Dyson, N. (1998) *Genes Dev.* 12, 2245–2262.
- (3) Helin, K. et al. (1993) *Genes Dev.* 7, 1850–1861.
- (4) Wu, C. et al. (1995) *Mol. Cell. Biol.* 15, 2536–2546.
- (5) Takahashi, Y. et al. (2000) *Genes Dev.* 14, 804–816.
- (6) Wu, L. et al. (2001) *Nature* 414, 457–462.
- (7) Gaubatz, S. et al. (2000) *Mol. Cell* 6, 729–735.
- (8) Hurford, R.K. et al. (1997) *Genes Dev.* 11, 1447–1463.
- (9) Tsai, K.Y. et al. (1998) *Mol. Cell* 2, 293–304.
- (10) Garcia, I. et al. (2000) *Cell Growth Differ.* 11, 91–98.
- (11) Lin, W.C. et al. (2001) *Genes Dev.* 15, 1833–1844.

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 blot analysis of extracts from HeLa cells, untransfected (-) or transfected with E2F-1 siRNA (+) to silence E2F-1 protein expression, using E2F-1 Antibody.



Western blot analysis of extracts from NIH/3T3, HeLa, SK-N-MC, and A673 cells, using E2F-1 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.

Recommended Antibody Dilutions:
Western blotting 1:1000

Companion Products:

- Phospho-p53 (Ser15) Antibody #9284
- Phospho-p53 (Ser15) (16G8) mAb #9286
- Phospho-p53 (Ser20) Antibody #9287
- Phospho-Rb (Ser795) Antibody #9301
- Phospho-Rb (Ser780) Antibody #9307
- Phospho-Rb (Ser807/811) Antibody #9308

Phototope®-HRP Western Detection System:
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

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Applications Key: W—Western IP—Immunoprecipitation IHC—Immunohistochemistry IC—Immunocytochemistry IF—Immunofluorescence 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 B—bovine 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 (for a polyclonal antibody) or 5% w/v nonfat dry milk (for a monoclonal antibody), 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 a polyclonal antibody) or 5% nonfat dry milk (for a monoclonal); for 20 ml, add 2 ml 10X TBS to 18 ml water, mix. Add 1.0 g BSA (for a polyclonal antibody) or 1.0 g nonfat dry milk (for a monoclonal antibody) and mix well. While stirring, add 20 µl Tween-20 (100%).
- Note: See the specific product's data sheet for the preferred blocking agent. In general, BSA is recommended for polyclonal antibodies; nonfat dry milk is recommended for monoclonal antibodies.*
- A10. ■ Phototope®-HRP Western Blot Detection System #7074 or #7076: Includes biotinylated protein marker, secondary anti-rabbit (#7074) or secondary anti-mouse (#7076) 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 Marker Detection Pack #7726 or #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 markers (#7726 or #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 cm²) 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.