Datasheet for ABIN487490

anti-E2F4 antibody

2 Images

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Overview

Quantity:	0.1 mg
Target:	E2F4
Reactivity:	Human, Mouse, Rat
Host:	Mouse
Clonality:	Monoclonal
Conjugate:	This E2F4 antibody is un-conjugated
Application:	Western Blotting (WB), Immunoprecipitation (IP), Immunofluorescence (IF)

Product Details

Immunogen:	Recombinant full-length Human E2F-4. Remarks: Hybridoma was established by fusion of Mouse myeloma cell NS-2 with Balb/cmouse splenocyte
Clone:	TFE42
Isotype:	IgG1
Specificity:	This antibody reacts with Human, Mouse and Rat E2F4 on Western blotting and Immunoprecipitation.
Cross-Reactivity (Details):	Species reactivity (tested):Human, Mouse and Rat.
Characteristics:	Synonyms: E2F-4, Transcription factor E2F4, p107/p130-binding, E2F transcription factor 4
Purification:	Protein-A Sepharose Chromatography.

Target Details

Target:	E2F4
Alternative Name:	E2F4 (E2F4 Products)
Background:	The E2F family of transcription factors regulates gene expression following heterodimerization with the DP family proteins, which promote transition to S phase from G1 phase. E2F activity is controlled by interactions with the retinoblastoma tumor suppressor family (pRB, p107 and p130). E2F proteins can be divided into three groups on homology and on certain functional characteristics. The first group containing E2F-1, E2F-2 and E2F-3 binds exclusively to pRB and has a cyclin A binding domain that mediates inhibition of DNA binding during entry into S phase. The second group is composed of E2F-4 and E2F-5, which preferentially bind to p107 and p130, although they can bind pRB as well. The last member is E2F-6. E2F-6 appears to act as a transcription repressor. E2F-4 and E2F-5 are functionally different from E2F-1 and E2F-3. E2F-1 and E2F-3 participate in cellular proliferation, whereas E2F-4 and E2F-5 are required for cell cycle arrest in G1 induced by cyclin-dependent kinase inhibitor p16INK4a.Synonyms: E2F transcription factor 4, E2F-4, Transcription factor E2F4, p107/p130-binding
Gene ID:	1874
UniProt:	Q16254
Pathways:	Cell Division Cycle, Mitotic G1-G1/S Phases, Regulation of Cell Size

Application Details

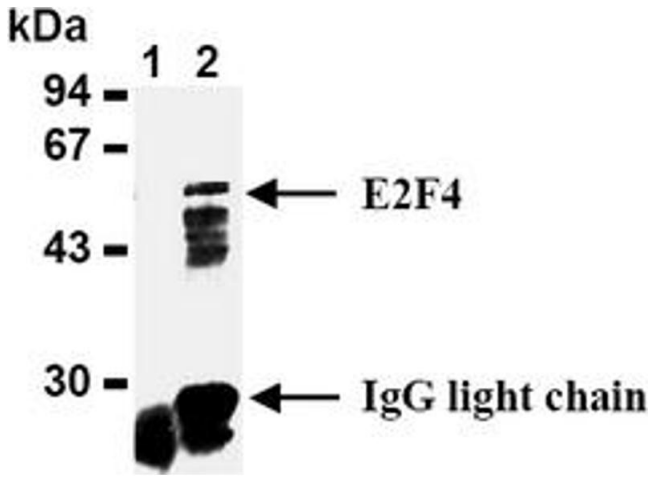
Application Notes:	Western Blot: 1-5 µg/mL for chemiluminescence detection system. Positive Controls: HL60, Raji, NIH/3T3, C2C12, Rat-1. Immunoprecipitation: 2 µg/200-300 µL of cell extract from 5 x 10^6 cells. Positive Control: HL60. It is reported that this monoclonal antibody can be used in Immunocytochemistry (Ref1). Detailed procedure is provided in Protocols. Other applications not tested. Optimal dilutions are dependent on conditions and should be determined by the user.
Protocol:	SDS-PAGE & Western Blotting1) Wash the cells 3 times with PBS and suspend with 10 volume of cold Lysis buffer (50 mMTris-HCl, pH 7. 2, 250 mM NaCl, 0. 1% NP-40, 2 mM EDTA, 10% glycerol) containingappropriate protease inhibitors. Incubate it at 4°C with rotating for 30 minutes, thensonicate briefly (up to 10 seconds). 2) Centrifuge the tube at 12,000 x g for 10 minutes at 4°C and transfer the supernatant toanother tube. Measure the protein concentration of the supernatant and add the Lysisbuffer to make 8 mg/mL solution. 3) Mix the sample with equal volume of Laemmli's sample buffer. 4) Boil the samples for 2 minutes and centrifuge. Load 10 µL of the sample per lane in a 1mm thick SDS-polyacrylamide gel for electrophoresis.

Application Details

	5) Blot the protein to a polyvinylidene difluoride (PVDF) membrane at 1 mA/cm2 for 1 hourin a semi-dry transfer system. (Transfer Buffer: 25 mM Tris, 190 mM glycine, 20% MeOH). See the manufacture's manual for the transfer procedure. 6) To reduce nonspecific binding, soak the membrane in 10% skimmed milk (in PBS, pH7. 2) for 1 hour at room temperature, or overnight at 4°C. 7) Incubate the membrane with primary antibody diluted with PBS, pH 7. 2 containing 1%skimmed milk as suggest in the APPLICATIONS for 1 hour at room temperature. (Theconcentration of antibody will depend on condition.)8) Wash the membrane with PBS (5 minutes x 6 times). 9) Incubate the membrane with the 1: 10000 POD-conjugated anti-mouse IgG diluted with1% skimmed milk (in PBS, pH 7. 2) for 1 hour at room temperature. 10) Wash the membrane with PBS-T (10 minutes x 3 times). 11) Wipe excess buffer from the membrane, then incubate it with appropriatechemiluminescence reagents for 1 minute. Remove extra reagent from the membrane bydabbing with a paper towel, and seal it in plastic wrap. 12) Remove extra reagent from the membrane by dabbing with paper towel, and seal it inplastic wrap. 13) Expose to an X-ray film in a dark room for 3 minutes. 14. Develop the film as usual. The conditions for exposure and development may vary. Positive Controls for Western blotting: HL60, Raji, NIH/3T3, C2C12, Rat-1. Immunoprecipitation1) Wash the cells 3 times with PBS and suspend with 10 volume of cold Lysis buffer (50 mMTris-HCl, pH 7. 2, 250 mM NaCl, 0. 1% NP-40, 2 mM EDTA, 10% glycerol) containingappropriate protease inhibitors. Incubate it at 4°C with rotating for 30 minutes, thensonicate briefly (up to 10 seconds). 2) Centrifuge the tube at 12,000 x g for 10 minutes at 4°C and transfer the supernatant toanother tube. 3) Add primary antibody as suggest in the APPLICATIONS into 300 µL of the supernatant. Mix well and incubate with gentle agitation for 30-120 minutes at 4°C. 4) Add 20 µL of 50% protein A agarose beads resuspended in the cold Lysis buffer. Mix
Restrictions:	For Research Use only

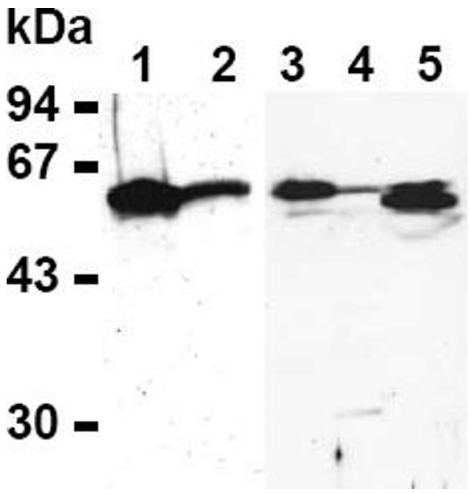
Handling

Concentration:	1.0 mg/mL
Buffer:	PBS, pH 7.2 containing 50 % Glycerol without preservatives.
Preservative:	Without preservative
Storage:	-20 °C
Storage Comment:	Store the antibody (in aliquots) at -20 °C. Avoid repeated freezing and thawing. Shelf life: one year from despatch.
Expiry Date:	12 months



Western Blotting

Image 1.



Western Blotting

Image 2.

