Datasheet for ABIN487487

anti-CHEK1 antibody

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Overview

Quantity:	0.1 mg
Target:	CHEK1
Reactivity:	Human
Host:	Mouse
Clonality:	Monoclonal
Conjugate:	This CHEK1 antibody is un-conjugated
Application:	Western Blotting (WB), Immunohistochemistry (Paraffin-embedded Sections) (IHC (p)), Immunoprecipitation (IP)

Product Details

Immunogen:	Human recombinant full length CHK1. Remarks: Hybridoma was established by fusion of Mouse myeloma cell NS-2 with Balb/cmouse splenocyte
Clone:	DCS-310
Isotype:	IgG2b
Specificity:	This antibody reacts with CHK1 (55 kDa) on Western blots.
Cross-Reactivity (Details):	Species reactivity (tested):Human.
Characteristics:	Synonyms: CHEK1, CHEK-1, Serine/threonine-protein kinase Chk1, CHK1 checkpoint homolog
Purification:	Protein-A Sepharose Chromatography.

Target Details

Target:	CHEK1
Alternative Name:	CHK1 (CHEK1 Products)
Background:	The DNA damage checkpoint is a signal transduction pathway that delays entry into mitosis following DNA damage. When DNA is damaged, Chk1 acts downstream of ATM to elicit appropriate responses such as cell cycle arrest. When activated by ATM, Chk1 phosphorylates serines 123, 178, 278, and 292 of the S phase-promoting CDC25A phosphatase, which accelerates IR (ionizing radiation)-induced degradation of CDC25A.Synonyms: CHEK-1, CHEK1, CHK1 checkpoint homolog, Serine/threonine-protein kinase Chk1
Gene ID:	1111
UniProt:	O14757
Pathways:	p53 Signaling, Apoptosis, Cell Division Cycle, DNA Damage Repair

Application Details

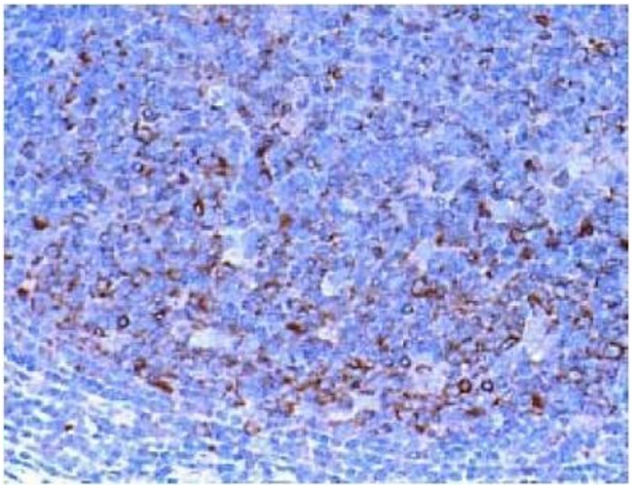
Application Notes:	Western Blot: 1 µg/mL mL for chemiluminescence detection system. Positive Controls: HeLa, MCF7, Raji Cells. Immunoprecipitation: 2 µg/200 µL of cell extract from 5x10^6 cells. Positive Control: Raji. Immunohistochemistry: 1 µg/mLHeat treatment is necessary for Paraffin Embedded Sections. Microwave oven: 2 times for 10 minutes each in citrate buffer (pH 6.5). Positive Control: Tonsil Tissue. 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. 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

Application Details

	1%skimmed milk as suggested in the APPLICATIONS for 1 hour at room temperature. (Theoptimal antibody concentration will depend on the experimental conditions.)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 (5 minutes x 6 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) Expose to an X-ray film in a dark room for 5 minutes. Develop the film as usual. Theconditions for exposure and development may vary. Positive Controls for Western blotting: HeLa, MCF7, RajiImmunoprecipitation1) 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 suggested in the APPLICATIONS into 200 µL of the supernatant. Mix well and incubate with gentle agitation for 30-120minutes at 4°C. Add 20 µL of 50% protein A agarose beads resuspended in the cold Lysisbuffer. Mix well and incubate with gentle agitation for 60 minutes at 4°C. 4) Wash the beads 3-5 times with ice-cold Lysis buffer (centrifuge the tube at 2,500 x g for10 seconds). 5) Resuspend the beads in 20 µL of Laemmli's sample buffer, boil for 3-5 minutes, andcentrifuge for 5 minutes. Use 10 µL/lane for the SDS-PAGE analysis. (See SDS-PAGE & Western blotting.)Positive Controls for immunoprecipitation: Raji.
Restrictions:	For Research Use only

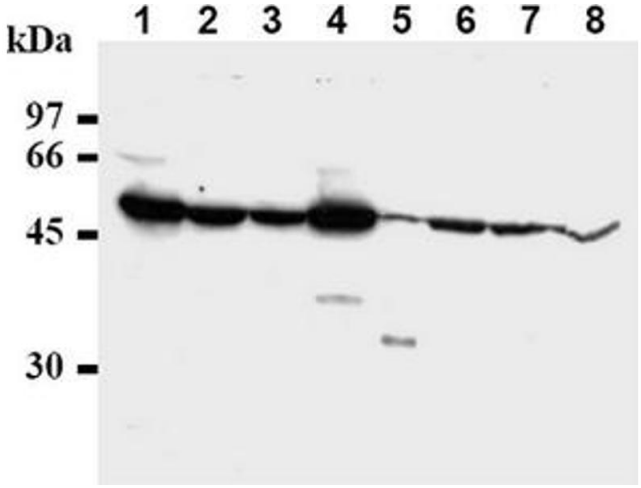
Handling

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 undiluted at -20 °C. Shelf life: one year from despatch.
Expiry Date:	12 months



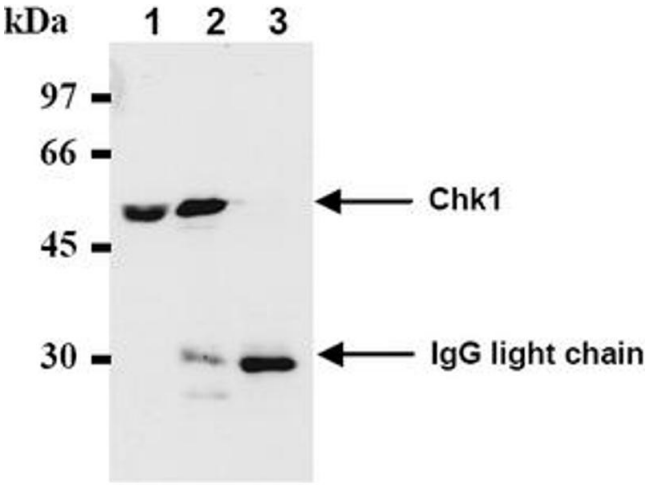
Immunohistochemistry

Image 1.



Western Blotting

Image 2.



Western Blotting

Image 3.

