Datasheet for ABIN492574

anti-RAD17 antibody

3 Images

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Overview

Quantity:	0.1 mg
Target:	RAD17
Reactivity:	Human, Mouse, Rat
Host:	Mouse
Clonality:	Monoclonal
Conjugate:	This RAD17 antibody is un-conjugated
Application:	Western Blotting (WB), Immunofluorescence (IF), Immunohistochemistry (Paraffin-embedded Sections) (IHC (p)), Immunoprecipitation (IP)

Product Details

Immunogen:	Recombinant full-length Human HRad17. Remarks: Hybridoma was established by fusion of mouse myeloma cell Sp2/0-Ag14 withBalb/c mouse splenocyte
Clone:	3-10E-010
Isotype:	IgG1
Specificity:	This antibody reacts with HRad17 (80 kDa) on Western blotting, Immunoprecipitation and Immunohistochemistry.
Cross-Reactivity (Details):	Species reactivity (tested):Human, Mouse and Rat.
Characteristics:	Synonyms: R24L, Cell cycle checkpoint protein RAD17, RF-C/activator 1 homolog
Purification:	Protein-A Sepharose Chromatography of hybridoma supernatant.

Target Details

Target:	RAD17
Alternative Name:	RAD17 (RAD17 Products)
Background:	HRad17 is the human homologue of the protein encoded by the yeast rad17 gene. The rad17 gene of Schizosaccharomyces pombe is one of several essential checkpoint components including rad1, rad3, rad9, rad17, hus1 and cut5/rad4, these are absolutely required for prevention of mitosis after DNA damage in fission yeast. HRad17 is localized to chromosome 5q12, 13.1, a region known to be deleted in a variety of human cancers which could lead to higher rate of mutation as well as increased sensitivity to radiation and chemotherapy.Synonyms: Cell cycle checkpoint protein RAD17, R24L, RF-C/activator 1 homolog
Gene ID:	5884
UniProt:	O75943

Application Details

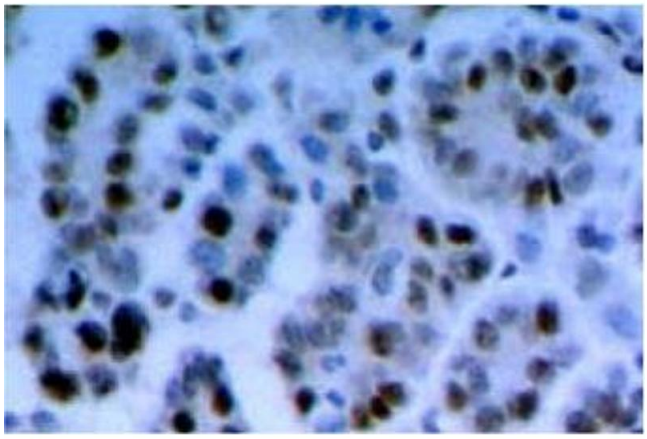
Application Notes:	Western blotting: 1 µg/mL for chemiluminescence detection system. Immunoprecipitation: 2 µg/200 µL of cell extract from 5x10^6 cells. Immunohistochemistry on Paraffin Embedded Sections: 1-5 µg/mL (Heat treatment isnecessary). Microwave oven: 2 times for 10 minutes each in 10 mM citrate buffer (pH 6.5). 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 cold Lysisbuffer to make 8 mg/mL solution. 3) Mix the sample with equal volume of Laemmli's sample buffer. 4) Boil the samples for 3 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 precise 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

Application Details

	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-T [0. 05% Tween-20 in PBS] (5 minutes x 3 times). 9) Incubate the membrane with the 1: 10,000 HRP-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 on the membrane, then incubate it with appropriatechemiluminescence reagent for 1 minute. Remove extra reagent from the membrane by dabbing with paper towel, and seal it inplastic wrap. 12) Expose to an X-ray film in a dark room for 3 minutes. Develop the film as usual. The condition for exposure and development may vary. Positive Controls: HeLa, HEp-2, NIH/3T3, PC12Immunoprecipitation1) 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 200 µL of the supernatant. Mix well and incubate with gentle agitation for 30-120 minutes at 4°C. Add 20 µL of 50%protein A agarose resuspended in the cold Lysis buffer. Mix well and incubate with gentleagitation for 60 minutes at 4°C. 4) Wash the beads 3-5 times with the cold Lysis buffer (centrifuge the tube at 2,500 x g for
Restrictions:	For Research Use only

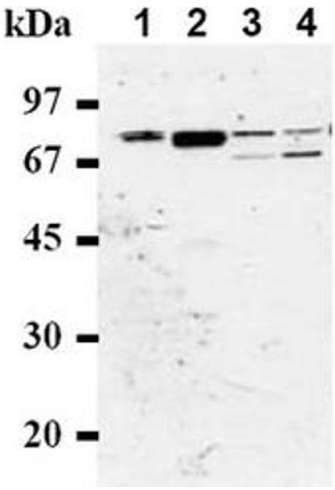
Handling

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



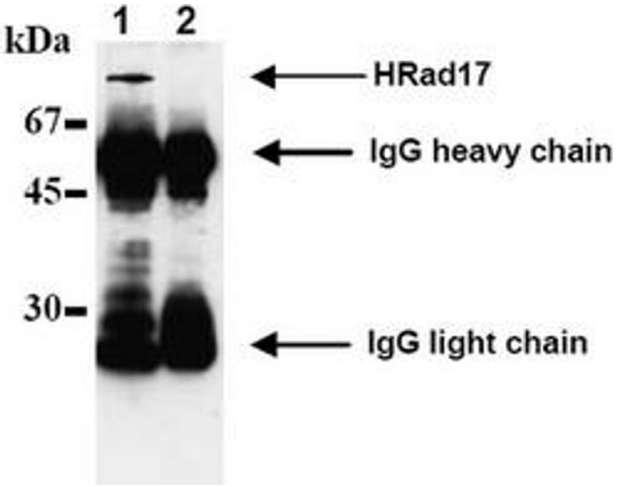
Immunohistochemistry

Image 1.



Western Blotting

Image 2.



Western Blotting

Image 3.

