Datasheet for ABIN492595

anti-CDC27 antibody (C-Term)

2 Images

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Overview

Quantity:	0.1 mg
Target:	CDC27
Binding Specificity:	AA 814-823, C-Term
Reactivity:	Human, Rat, Mouse, Cow, Dog, Xenopus laevis
Host:	Mouse
Clonality:	Monoclonal
Conjugate:	This CDC27 antibody is un-conjugated
Application:	Western Blotting (WB), Immunoprecipitation (IP), Immunohistochemistry (Paraffin-embedded Sections) (IHC (p)), Enzyme Immunoassay (EIA)

Product Details

Immunogen:	Peptide corresponding to C-terminal of Human CDC27 (aa 814-823).
Clone:	AF3-1
Isotype:	IgG2b
Specificity:	This antibody reacts with 97 kDa membrane protein CDC27 on Western blotting, Immunoprecipitation and Immunohistochemistry.
Cross-Reactivity (Details):	Species reactivity (expected):Bovine, Canine and Xenopus leavis. Species reactivity (tested):Human, Mouse, Rat.
Characteristics:	Synonyms: CDC27Hs, H-NUC, D0S1430E, D17S978E, Cell division cycle protein 27 homolog
Purification:	Protein-A Agarose Chromatography of hybridoma supernatant.

Target Details

Target:	CDC27
Alternative Name:	CDC27 (CDC27 Products)
Background:	CDC16, CDC27 and CDC34 are components of the APC (anaphase-promoting complex) that also play a role as components of the ubiquitin- conjugating E3 enzyme. A 20S complex containing CDC27 and CDC16 catalyzes the mitosis-specific conjugation of ubiquitin to cyclin B, resulting in cyclin B/Cdk complex degradation. CDC27 is therefore required for progression of the cell cycle, where it executes essential mitotic functions near the metaphase/anaphase transition. CDC27 is ~97 kDa and is localized to the centrosome and mitotic spindle.Synonyms: CDC27Hs, Cell division cycle protein 27 homolog, D0S1430E, D17S978E, H-NUC
Gene ID:	996
UniProt:	P30260

Application Details

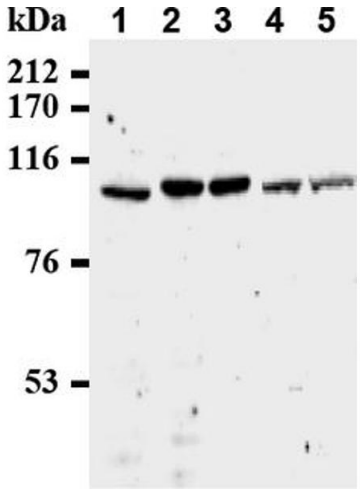
Application Notes:	Western blotting: 1 µg/mL for chemiluminescence detection system. Positive Controls: Jurkat, Raji, K562, HL-60. Immunoprecipitation: 2 µg/200 µL of cell extract. Positive Control: Raji. Immunohistochemistry on Paraffin Embedded Sections: 10 µg/mL. (Heat treatment is necessary)Autoclave: 10 minutes at 110 °C in 10 mM citrate buffer (pH 6.5). Positive Control: Tonsil. 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 RT. 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. 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 condition for exposure and development may vary. Positive Controls: Jurkat, Raji, K562, HL-60, MCF-7Immunoprecipitation1) Wash the cells 3 x 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.
Restrictions:	For Research Use only

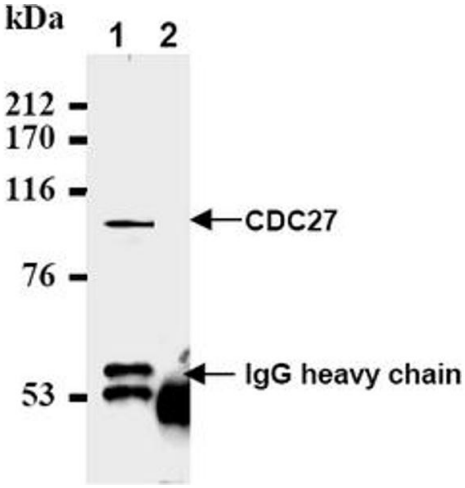
Handling

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.