OriGene Technologies, Inc.
9620 Medical Center Drive, Ste 200
Rockville, MD 20850
UNITED STATES
Phone: +1-888-267-4436
Fax: +1-301-340-8606
techsupport@origene.com

OriGene Technologies GmbH
Schillerstr. 5
32052 Herford
GERMANY
Phone: +49-5221-34606-0
Fax: +49-5221-34606-11
info-de@origene.com

AM20032AF-N Monoclonal Antibody to CHK2 - Azide Free

Alternate names: CHEK-2, CHEK2, CHK-2, CHK2 checkpoint homolog, Cds1, RAD53, Serine/threonine-protein kinase Chk2

Quantity: 0.1 mg

Concentration: 1.0 mg/ml

Background: Checkpoint kinase 2 (Chk2), also known as Cds1, is a 61 kDa nuclear protein that functions as a checkpoint kinase to regulate the cell cycle progression following DNA damage. Chk2 inhibits CDC2 by inactivating CDC25, the phosphatase that normally activates CDC2. Other targets for Chk2 include the tumor suppressors BRCA1 and p53, which it stabilizes by phosphorylation of Ser20. Chk2 is itself phosphorylated and activated by ATM following DNA damage. Defects in Chk2 contribute to the development of human cancers, and implicate Chk2 as a candidate tumor suppressor and an attractive target for drug discovery.

Uniprot ID: O96017

NCBI: NP_001005735

GeneID: 11200

Host / Isotype: Mouse / IgG2a

Clone: DCS-273

Immunogen: Full-length Human Chk2 fusion protein.
Remarks: Hybridoma was established by fusion of Mouse myeloma cell NS-2 with Balb/c mouse splenocyte

Format: **State:** Liquid purified IgG fraction.
Purification: Protein-A Sepharose Chromatography.
Buffer System: PBS, pH 7.2 containing 50% Glycerol without preservatives.

Applications: **Western Blot:** 1 µg/ml.
Positive Control: HeLa.
Immunoprecipitation: 3 µg/200-300 µL of cell extract.
Positive Control: HeLa.
Immunohistochemistry: 1-5 µg/mL
Heat 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.

Specificity: This antibody reacts with Human Chk2.

Species Reactivity: **Tested:** Human.

Add. Information: This product was originally produced by MBL International.

Storage:

Store the antibody (in aliquots) at -20°C.
Avoid repeated freezing and thawing.
Shelf life: one year from despatch.

General Readings:

Clone DCS-273 is used in these references:
1. Syljuäsen, R.G., et al., Cancer Res. 66, 10253-10257 (2006)
2. Berger, M., et al., Mol. Cell Biol. 25, 5380-5388 (2005)
3. Latella, L., et al., Mol. Cell Biol. 24, 6350-6361 (2004)
4. Louria-Hayon, I., et al., J. Biol. Chem. 278, 33134-33141 (2003)
5. Lukas, C., et al., Cancer Res. 61, 4990-4993 (2001)

Protocols:

SDS-PAGE & Western Blotting

1) Wash the cells 3 times with PBS and suspend with 10 volume of cold Lysis buffer (50 mM Tris-HCl, pH 7.2, 250 mM NaCl, 0.1% NP-40, 2 mM EDTA, 10% glycerol) containing appropriate protease inhibitors. Incubate it at 4°C with rotating for 30 minutes, then sonicate briefly (up to 10 seconds).
2) Centrifuge the tube at 12,000 x g for 10 minutes at 4°C and transfer the supernatant to another tube. Measure the protein concentration of the supernatant and add the Lysis buffer 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 1 mm thick SDS-polyacrylamide gel for electrophoresis.
5) Blot the protein to a polyvinylidene difluoride (PVDF) membrane at 1 mA/cm2 for 1 hour in 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, pH 7.2) for 1 hour at room temperature, or overnight at 4°C.
7) Incubate the membrane with the anti-Chk2 (DCS-273) monoclonal antibody (1 µg/mL) diluted with 1% skimmed milk (in PBS, pH 7.2) for 1 hour at room temperature.
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 with 1% 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 appropriate chemiluminescence reagents for 1 minute. Remove extra reagent from the membrane by dabbing 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. The conditions for exposure and development may vary.
Positive Controls for Western blotting: HeLa

Immunoprecipitation

1) Wash the cells 3 times with PBS and suspend with 10 volume of cold Lysis buffer (50 mM Tris-HCl, pH 7.2, 250 mM NaCl, 0.1% NP-40, 2 mM EDTA, 10% glycerol) containing appropriate protease inhibitors. Incubate it at 4°C with rotating for 30 minutes, then sonicate briefly (up to 10 seconds).
2) Centrifuge the tube at 12,000 x g for 10 minutes at 4°C and transfer the supernatant to another tube.
3) Add 3 µg of the anti-Chk2 (DCS-273) monoclonal

antibody into 250 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 beads resuspended in the Lysis buffer. 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 for 10 seconds).
5) Resuspend the beads in 20 µL of Laemmli's sample buffer, boil for 3-5 minutes, and centrifuge for 5 minutes. Use 10 µL/lane for the SDS-PAGE analysis.
(See **SDS-PAGE & Western blotting.)**
Positive Controls for immunoprecipitation: HeLa.

Immunohistochemical Staining for Paraffin-Embedded Sections: SAB method
1) Deparaffinize the sections with Xylene 3 times for 3-5 minutes each.
2) Wash the slides with Ethanol 3 times for 3-5 minutes each.
3) Wash the slides with PBS 3 times for 3-5 minutes each.
4) Heat treatment
Heat treatment by microwave oven: Place the slides put on staining basket in 500 mL beaker with 500 mL citrate buffer (pH 6.5). Cover the beaker with plastic wrap, then process the slides 2 times for 10 minutes each at 500 W with microwave oven. Let the slides cool down in the beaker at room temperature for about 40 minutes.
5) Remove the slides from the citrate buffer and cover each section with 3% H2O2 for 10 minutes at room temperature to block endogenous peroxidase activity. Wash 3 times in PBS for 5 minutes each.
6) Remove the slides from PBS, wipe gently around each section and cover tissues with Protein Blocking Agent for 5 minutes to block non-specific antibody staining. Do not wash.
7) Tip off the blocking buffer, wipe gently around each ection and cover tissues with the anti-Chk2 (DCS-273) Monoclonal antibody diluted with PBS containing 1% BSA (1-5 µg/mL).
8) Incubate the sections for 1 hour at room temperature.
9) Wash the slides 3 times in PBS for 5 minutes each.
10) Wipe gently around each section and cover tissues with Polyvalent Biotinylated Antibody. Incubate for 10 minutes at room temperature. Wash as in step 9.
11) Wipe gently around each section and cover tissues with Streptavidin-Peroxidase. Incubate for 10 minutes at room temperature. Wash as in step 9.
12) Visualize by reacting for 10-20 minutes with substrate solution containing 7.5 mg DAB, 40 µL of 30% H2O2 in 150 mL PBS. *DAB is a suspected carcinogen and must be handled with care. Always wear gloves.
13) Wash the slides in water for 5 minutes.
14) Counter stain in hematoxylin for 1 minute, wash the slides 3 times in water for 5 minutes each, and then immerse the slides in PBS for 5 minutes. Dehydrate by immersing in Ethanol 3 times for 3 minutes each, followed by immersing in Xylene 3 times for 3 minutes each.
15) Now ready for mounting.
Positive Controls for Immunohistochemistry: Tonsil, Intestine.

Pictures:

Figure 1. AM20032AF-N CHK2 antibody Immunohistochemical staining of Human Intestine Paraffin Embedded Section.

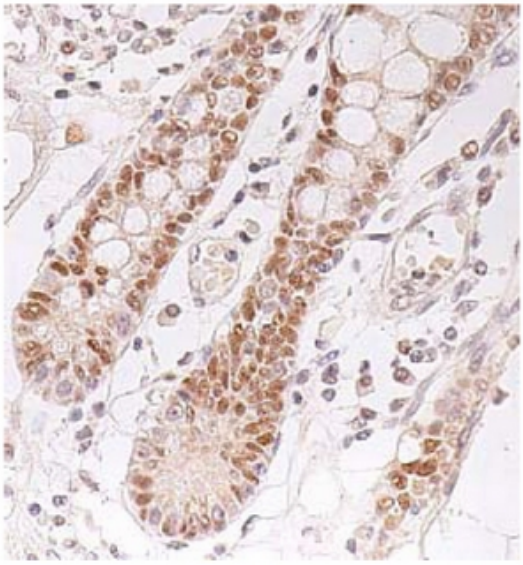

Figure 2. Western blot analysis of Chk2 expression in HeLa cells (Lane 1), NIH/3T3 cells (Lane 2) and Rat-1 cells (Lane 3) using CHK2 antibody AM20032AF-N.

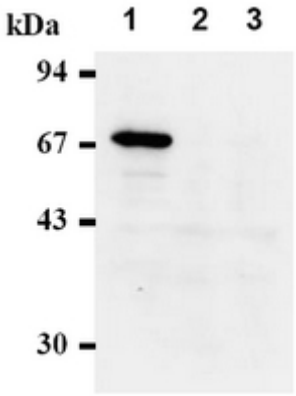

Figure 3. Immunoprecipitation of Chk2 from HeLa cells with normal Mouse IgG (Lane 1) or AM20032AF-N (Lane 2). After immunoprecipitated with the antibody, immunocomplex was resolved on SDS-PAGE and immunoblotted with CHK2 antibody (AM20032AF-N).