BM4507

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Monoclonal Antibody to BubR1 - Purified

Catalog No.: BM4507

Quantity: 0.1 ml

Concentration: 3.0 mg/ml

Background: BUBR1 is a predicted 1050 amino acid mitotic checkpoint kinase which also controls chromosome segregation. It contains 2 domains: CD1 directs kinetochore localization and binding to Bub3, and CD2 contains the kinase domain. Between CD1 and CD2, the BUB1B protein has a putative nuclear localization signal. BUBR1 contains a putative cyclin destruction box that can target proteins for degradation by proteosomes during mitosis.

Host / Isotype: Mouse / IgG1

Recommended Isotype Controls: SM10P (for use in human samples), AM03095PU-N

Clone: P10.8G1.G11.H12

Immunogen: N-terminal tagged GST fusion recombinant fragment, corresponding to amino acids 1-350 of Human BUBR1

Format:
State: Liquid purified Ig fraction.
Purification: DEAE chromatography.
Buffer System: PBS with 0.09% sodium azide as preservative.

Applications: Suitable for Immunoprecipitation (3 µg), Immunofluorescence (1/1500) and Western blot (1/500-1/1000, 50 µg of Hela lysate).
Any human cell line can be used as a positive control. Subcellular localization of antigens are only detected in mitotic cells.
Other applications not tested. Optimal dilutions are dependent on conditions and should be determined by the user.

Specificity: HumanBUBR1.
Not tested for reactivity with rodents or other vertebrates.

Storage: Store the antibody at 2-8°C for one month or (in small aliquots) at -20°C for longer.
Avoid repeated freezing and thawing.
Shelf life: One year from despatch.

General Readings:
1. Zhou J, Panda D, Landen JW, Wilson L, Joshi HC. Minor alteration of microtubule dynamics causes loss of tension across kinetochore pairs and activates the spindle checkpoint. J Biol Chem. 2002 May 10;277(19):17200-8. Epub 2002 Feb 25. PubMed PMID: 11864974.
2. Liu ST, Hittle JC, Jablonski SA, Campbell MS, Yoda K, Yen TJ. Human CENP-I specifies localization of CENP-F, MAD1 and MAD2 to kinetochores and is essential for mitosis. Nat Cell Biol. 2003 Apr;5(4):341-5. PubMed PMID: 12640463.

Protocols:
Immunofluorescence protocol - Formaldehyde fixation
Collect cells from T.c.unit and remove media from petri dish using suction.

Wash with 1x PBS and remove.
Incubate cells in pre-warm (37°C) Para-Formaldehyde for 12 minutes at room temperature on an orbital shaker.
Remove PFA and incubate in 0.5% Triton X-IOO in 1x PBS for 5 minutes at room temperature.
Prepare blocking reagent, this is also the antibody diluent.
Wash cells 2x with 1x PBS at room temperature, for 4 minutes/wash on an orbital shaker.
Block with 1 % NCS and 1x PBS for 30 minutes at room temperature.
Prepare primary antibodies (50µl/coverslip) and moist staining chambers.
Wash cells 2x with lx PBS at room temperature and air dry briefly.
Incubate with primary antibody for 1 hr at room temperature in the dark in staining chambers. During this time prepare the secondary antibody.
Wash cells 5x with 1x PBS (5 beaker changes/5 counts in each beaker)
Incubate with secondary antibody for 1 hour at room temperature in the dark in staining chambers.
Wash cells 5x with 1x PBS.
Mount in Dapi.
Solutions (prepare fresh the same day of staining):
1x Phosphate buffered saline.
Blocking reagent: 1% NCS in 1x PBS (use fresh l0x PBS).
Fixation solution: 3.5% Para formaldehyde.
1.75g PFA in 20 ml d.H20 plus 5 drops 1M NaOH. Stir on a hot plate at 50-60°C until dissolved. Add 4 drops IN HCI and check pH indicator strip. PH should be 7.4. Complete volume with d.H20 to 25ml and add 25ml 2xPBS. Check pH before adding to cover slips.

Immunofluorescence protocol - Methanol/acetone fixation

Collect cells from T.C.unit and remove media from petri dish using suction.
Wash with 1x PBS and remove.
Fix cells with cold methanol: acetone 1: 1 for 10 minutes on ice.
Prepare blocking reagent, this is also the diluent for the antibodies.
Remove fixative and wash cells 3x with Ix PBS at RT, for 4 minutes/wash on orbital shaker.
Block with 1% NCS and Ix PBS for 30 minutes at RT.
Prepare primary antibodies (50µl/coverslip) and moist staining chambers.
Wash cells 2x with 1 x PBS at RT and air dry for approximately 7 minutes.
Incubate with primary antibody for 1 hr at RT in the dark in staining chambers. During this time prepare secondary antibody.
Wash cells 5x with 1x PBS (5 beaker changes/5 counts in each beaker)
Incubate with secondary antibody for 1 hr at R T in the dark in staining chambers.
Wash cells 5x with 1x PBS.
Mount in Dapi.
Solutions (prepare fresh the same day of staining):
1x Phosphate buffered saline.
Blocking reagent: 1% NCS in 1x PBS (use fresh 10x PBS).
Fixation solution: methanol:acetone 1: 1 ice cold.

Western Blotting Protocol

Transfer gel to PDVF or nitrocellulose membrane
Place membrane in plastic tray in blocking buffer for one hour with agitation
Rinse in wash buffer
Incubate in wash buffer plus primary antibody for one hour
Wash 6 X 5 minutes with wash buffer
Incubate in wash buffer plus secondary antibody for one hour
Wash 6X 5 minutes with wash buffer
Detect (e.g. ECL, Amersham according to manufacturers instructions)
Wash buffer: PBS + 0.1% Tween 20

Blocking buffer: Wash buffer + 5% dried milk powder
The concentration of antibodies used depends on each antibody, the amount of antigen and the detection method used. Generally, dilution is in the range of a few hundred times dilution to a few thousand times dilution, but usually has to be determined empirically.