OriGene Technologies Inc.

9620 Medical Center Drive, Ste 200
Rockville, MD 20850
UNITED STATES
Phone: +1-858-888-7900
Fax: +1-858-888-7904
US-info@acris-antibodies.com

OriGene EU

Acris Antibodies GmbH
Schillerstr. 5
32052 Herford
GERMANY
Phone: +49-5221-34606-0
Fax: +49-5221-34606-11
info@acris-antibodies.com

Monoclonal Antibody to Mitotic Phosphoproteins pSer/Thr-Pro - Ascites

Catalog No.: AM32698SU-N

Quantity: 0.1 ml

Background: The life cycle of a eukaryotic cell consists of various phases, two of which can easily be identified. Firstly, during mitosis (M phase), in which the cell divides into two identical daughter cells, chromosome condensation and spindle formation are microscopically visible. Secondly, in S phase the DNA of a cell is replicated, a process that can be detected using biochemical techniques. In between the M and S phase two gap phases occur: the G1 phase, the gap between mitosis and the start of DNA replication, and G2 phase, the gap between completion of DNA replication and the onset of mitosis. From G1 phase a cell can leave the cell cycle and enter G0, a 'quiescent' phase. Regulation of the cell cycle predominantly occurs at three major control points, which govern the transition from G0 to G1, from G1 to S and from G2 to M phase. Upon entry into M (Mitosis) phase, many proteins are phosphorylated either directly or indirectly by M phase promoting factor (MPF).

Host / Isotype: Mouse / IgG2a

Clone: CC-3

Immunogen: Cell homogenates from pharyngeal chick embryos (Ref.1)

Format:
State: Liquid Ascites
Preservatives: 0.05% Sodium Azide
Stabilizers: 30% Glycerol

Applications:
Immunoblot Analysis: 1/1000-1/5000 dilutions of this lot detected phosphorylated mitotic proteins in RIPA lysates from nocodazole treated HeLa cells.
Immunocytochemistry: This antibody has been reported by an independent laboratory to detect phosphorylated mitotic proteins (Ref.2).
Immunoprecipitation: This antibody has been reported by an independent laboratory to immunoprecipitate phosphorylated mitotic proteins.
Other applications not tested. Optimal dilutions are dependent on conditions and should be determined by the user.

Specificity: This antibody recognizes a variety of proteins that are phosphorylated during mitosis (Ref.2,3).

Species Reactivity: **Tested:** Human, Mouse, Rat and Chicken.

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

General Readings:
1. Thibodeau, A. and Vincent, M., Exp. Cell Res. 95: 145-153, 1991.
2. Albert, A.L. et al., J. Cell Sci. 112: 2493-2500, 1999.

3. Albert, A.L. et al., BMC Cell Biol. 5: 22, 2004.

Protocols:

Immunoblot

1. Perform SDS-polyacrylamide gel electrophoresis (SDS-PAGE) on a cell lysate sample (cell lysis buffer: 50mM Tris-HCl, pH 7.4; 1% NP-40; 0.25% sodium deoxycholate; 150mM NaCl; 1mM EDTA; 1mM PMSF; 1µg/ml each aprotinin, leupeptin, pepstatin; 1mM Na3VO4, 1mM NaF) and transfer the proteins to nitrocellulose. Wash the blotted nitrocellulose twice with water.
2. Block the blotted nitrocellulose in freshly prepared 5% nonfat dry milk in TBS with 0.05% Tween®-20 (TBST-MLK) for 30 minutes at room temperature with constant agitation.
3. Incubate the nitrocellulose with a 1/1000-1/5000 dilution of anti-phospho-Ser/Thr-Pro, clone CC-3, diluted in freshly prepared TBST-MLK overnight with agitation at 4°C.
4. Wash the nitrocellulose twice with water.
5. Incubate the nitrocellulose in the secondary reagent of choice (a goat anti-mouse HRP conjugated IgG, 1/3000 dilution was used) in TBST-MLK for 1.5 hours with agitation at room temperature.
6. Wash the nitrocellulose twice with water.
7. Wash the nitrocellulose in TBS-0.05% Tween®-20 for 3-5 minutes.
8. Rinse the nitrocellulose in 4-5 changes of water.
9. Use detection method of choice (enhanced chemiluminescence was used).

Pictures:

Immunoblot Analysis: Untreated HeLa (Lane 1), nocodazole treated (Lane 2) and lambda protein phosphatase treated following nocodazole treatment (Lane 3) cell lysates were resolved by electrophoresis, transferred to nitrocellulose and probed with anti-Phospho-Ser/Thr-Pro Cat.-No AM32698SU-N (clone CC-3, 1/1000 dilution). Proteins were visualized using a Goat anti-Mouse secondary antibody conjugated to HRP and a chemiluminescence detection system.