

Affinity-purified Goat Anti-human/mouse Cyclin A2 Antibody

ORDERING INFORMATION

Catalog Number: AF5999

Lot Number: CDGX01

Size: 100 µg

Specificity: human/mouse Cyclin A2

Immunogen: *E. coli*-derived rhCyclin A2
(aa 73 - 199)

Ig Type: goat IgG

Application: Western blot

Background

Cyclin A2 (also CCNA2) is a member of the cyclin AB subfamily, cyclin family of proteins. Cyclin A2 is ubiquitously expressed, in contrast to Cyclin A1 that only appears in late pachytene spermatocytes. It associates with CDK1 during G2 and M phase, and CDK2 in S phase, of the cell cycle. Binding to SCAPER restricts Cyclin A2 to the cytoplasm. In both compartments, it provides substrate specificity to a phosphorylating complex that promotes entry into, and progression through, mitosis. Human Cyclin A2 is 432 amino acids (aa) in length. It contains two cyclin box folds (aa 210 - 299 and 309 - 430) and four acetylation sites that induce molecule turnover (Lys54/68/95/112). Over aa 73 - 199, human Cyclin A2 shares 83% aa identity with mouse Cyclin A2.

Preparation

Goat antibodies were raised against purified, *E. coli*-derived recombinant human Cyclin A2 (rhCyclin A2; aa 73 - 199; Accession # P20248). Polyclonal antibody was affinity-purified on a column derivatized with the recombinant protein and further purified by isolating the IgG fraction.

Formulation

Lyophilized from a 0.2 µm filtered solution in phosphate-buffered saline (PBS) with 5% trehalose.

Reconstitution

Reconstitute in PBS containing 0.02% Na₂S₂O₃.

Storage

Lyophilized samples are stable for twelve months from date of receipt when stored at -20° C to -70° C. Upon reconstitution, the antibody can be stored at 2° - 8° C for 1 month without detectable loss of activity. Reconstituted antibody can also be aliquotted and stored frozen at -20° C to -70° C in a manual defrost freezer for six months without detectable loss of activity. **Avoid repeated freeze-thaw cycles.**

Specificity

This antibody detects endogenous human Cyclin A2 in Western blot with an approximate molecular weight of 60 kDa.

Application

Western blot - An antibody concentration of 0.5 µg/mL is recommended.

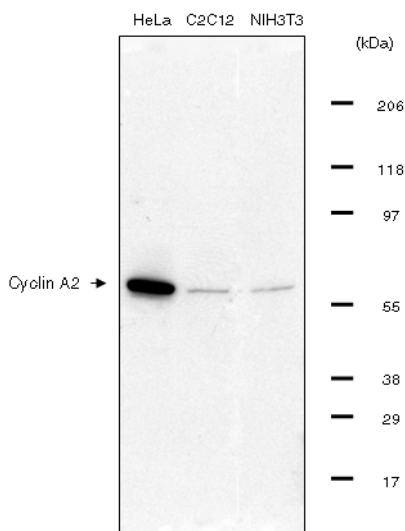
Protocols for Immunoblotting

Blotting Buffer	Blocking Solution	Antibody Solution
25 mM Tris, pH 7.4	5% nonfat dry milk	5% nonfat dry milk
0.15 M NaCl	in Blotting Buffer	in Blotting Buffer
0.1% Tween® 20	Adjust pH to 7.4	Adjust pH to 7.4

1. Transfer the electrophoresed proteins to a PVDF membrane and incubate the membrane for 1 hour at room temperature in Blocking Solution.
2. Incubate the membrane 1 hour at room temperature in Antibody Solution containing 0.5 µg/mL goat anti-human Cyclin A2.
3. Wash the membrane at room temperature for 30 minutes with 3 or more changes of Blotting Buffer. Changing the membrane containers often reduces background.
4. Incubate the membrane at room temperature for 1 hour in Antibody Solution containing a 1:2000 dilution of HRP-conjugated donkey anti-goat IgG (R&D Systems, Catalog # HAF109).
5. Wash the membrane for 30 minutes with 3 or more changes of Blotting Buffer.
6. Detect with chemiluminescent detection reagents.

Cell lysates for Western blottings - A single plate (150 mm) of exponentially growing cells is washed twice in cold PBS. 1 mL of boiling 1% SDS lysis buffer (1% SDS, 10 mM Tris-HCl, pH 7.4, 1 mM sodium ortho-vanadate) is added to the plate. The plate is then scraped and the lysis is collected, sonicated and quantified. 30 µg of cellular protein is added to an equal amount of 2x SDS loading buffer. Samples are then boiled for 5 minutes and run on a SDS-PAGE gel.

Optimal dilutions should be determined by each laboratory for each application.



Detection of Cyclin A2 with AF5999.

30 µg of whole cell extracts from HeLa, C2C12, and NIH3T3 cells were prepared, resolved by SDS-PAGE, and transferred to a PVDF membrane. The membrane was immunoblotted with 0.5 µg/mL goat anti-Cyclin A2 antibody, as described in *Protocols for Immunoblotting*.