IOTest® Anti-Cyclin A2-FITC

PN A22327 – 100 tests – 20 µL / test – Clone 11B2G3

For Research Use Only. Not for use in diagnostic procedures.

SPECIFICITY

Cyclin A2 is a 432 amino-acid long protein of 48 kDa that shares with other cyclins a homologous region of about 100 residues, known as the cyclin box, from residue 208 to 306, and a destruction box (D-box) from residue 47 to residue 57. The monoclonal antibody (mAb) 11B2G3 recognizes the human cyclin A2.

The cell cycle control system is based on two key families of proteins: cyclins and cyclin-dependent protein kinases (CDKs). Cyclins are characterized by their rapid accumulation and degradation during the cell cycle phases and are so called as they undergo a cycle of synthesis and degradation during each division cycle. Contrary to cyclins, CDK levels are relatively constant during the cell cycle.

Cyclins control CDKs activation by forming a cyclin-CDK complex able to phosphorylate selected proteins on serines and threonines, which induces downstream processes (1). Kinase activity of the cyclin-CDK complex is terminated by cyclin degradation via the ubiquitin-dependent proteolysis pathway.

In mammalian cells, there are two main classes of cyclins: mitotic cyclins (cyclin A and B, the first isolated) and G1 cyclins (cyclin D and E). Cyclin D-CDK4 and cyclin D-CDK6 complexes regulate the passage from G0 of G1. Cyclin E-CDK2 complex is important for G1 progression and G1 to S transition. Cyclin A can activate two different CDKs: CDK2 for the G1-S phase transition, and CDK1 (also called CDC2) for the G2-M transition. Finally, cyclin B-CDK1 regulates mitosis (2, 3). Thus, although cyclin B is the main cyclin for the mitosis, cyclin A is needed for progression through early mitosis. (4).

In humans, there are two A-type cyclins – an embryonic-specific cyclin A1 and a somatic cyclin A2. Cyclin A1 is only expressed in meiosis and very early embryos, whereas cyclin A2 is present in proliferating somatic cells (5). The mAb 11B2G3 recognizes cyclin A2.

Cyclin A-CDK2 and cyclin E-CDK2 complexes initiate DNA replication by first assembling a replication complex (RC) on chromatin during G1 phase. RC, composed of the loading factor (cdc6), the origin recognition complex (ORC) and the minichromosome maintenance (MCM) complex, is bound to the DNA replication origins. RC elements phosphorylation by cyclin A-CDK2 fires DNA replication (3, 6). Furthermore, cyclin A-CDK2 complex, present during all the S phase, inhibits the assembly of new RC and thereby replication by maintaining the MCM complex phosphorylated until cyclin A is abruptly degraded in the early phase of mitosis (before metaphase), as the phosphorylated MCM complex cannot bind to DNA (7). It has been shown that cyclin A- and cyclin E-CDK complexes shuttle between nucleus and cytoplasm and they have been implicated as regulator of centrosome replication (8). A recent study shows that cyclin A expression correlates with phosphorylated H2AX (9).
Tumor cells are characterized by genetic instability, a factor to acquire quickly new potentials. Although the mechanisms behind genetic instability vary considerably between tumors, all tumor cells must fail to detect and/or correctly react to the genetic damage. Understand the causes of unbalance between activated cyclins and regulating proteins like p53, p21 is another challenge to understand tumorigenesis (5).

REAGENT

IOTest Anti-Cyclin A2-FITC Conjugated Antibody
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Clone	11B2G3
Isotype	IgG1, mouse
Immunogen	Purified cyclin A2 (from HeLa cells)
Source	Ascites fluid
Purification	Affinity chromatography on Protein A
Conjugation	fluorescein isothiocyanate (FITC) is conjugated at 3 – 10 moles of FITC per mole of Ig.

Excitation wavelength: 488 nm
Maximum emission wavelength: 525 nm
Main emission color: Green
Buffer 2 mg/mL bovine serum albumin in phosphate-buffered saline containing 0.1% sodium azide.

APPLICATION

This reagent, designed for use in flow cytometry, may be used for studying cyclin A2 expression during cell cycle, in particular during S phase where its level increases and peaks at G2, and for distinguishing mitotic cells (cyclin A2 negative) from the G2 cells (cyclin A2 positive). Cyclin A2 is a marker of actively proliferating cells. It may be useful to study cyclin A2 expression in tumor cells.

STATEMENT OF WARNINGS

1. This reagent contains 0.1% sodium azide. Sodium azide under acid conditions yields hydrazoic acid, an extremely toxic compound. Azide compounds should be flushed with running water while being discarded. These precautions are recommended to avoid deposits in metal piping in which explosive conditions can develop. If skin or eye contact occurs, wash excessively with water.
2. Specimens, samples and all material coming in contact with them should be handled as if capable of transmitting infection and disposed of with proper precautions.
3. Never pipet by mouth and avoid contact of samples with skin and mucous membranes
4. Do not use antibody beyond the expiration date on the label.
5. Do not expose reagents to strong light during storage or incubation.
6. Avoid microbial contamination of reagents or incorrect results might occur.

STORAGE CONDITIONS AND STABILITY

This reagent is stable up to the expiration date when stored at 2 – 8°C. Do not freeze. Minimize exposure to light.

REAGENT PREPARATION

No reconstitution is necessary. This monoclonal antibody may be used directly from the vial. Bring reagent to 18 – 25°C prior to use.

PROCEDURE

Permeabilization using cold methanol

1. Take a volume of cell culture to have at least 1 x 10^6 cells.
2. Wash the cells with PBS and discard the supernatant.
3. While vortexing, add 1 mL per 1 x 10^6 cells of cold methanol (–20°C).
4. Incubate for 10 minutes on ice.
5. Centrifuge at 300 x g for 10 min. at room temperature (18 – 25 °C) and discard the supernatant.
6. Resuspend the pellet and add 3 mL of PBS containing 0.5% BSA and 0.05% NaN_3.
7. Centrifuge at 300 x g for 10 min. at room temperature.
8. Discard the supernatant and resuspend the pellet in PBS/BSA/NaN_3 to 5 x 10^6 cells/mL.

Staining

1. Pipet 100 µL of cell suspension at 5 x 10^6 cells/mL into two tubes (control and test).
2. Add 20 µL of anti-cyclin A2-FITC to the test tube and 20 µL of FITC-conjugated isotypic control (IM0639) to the control tube.
3. Add 20 µL of 7-AAD (IM3422) into each tube.
4. Mix gently and incubate for 20 min. at room temperature (18 – 25°C) in the dark.
5. Wash the preparation by adding 3 mL of PBS/BSA/NaN_3.
6. Centrifuge at 300 x g for 10 min. at room temperature.
7. Discard the supernatant and resuspend the pellet in 700 µL of PBS/BSA/NaN_3.

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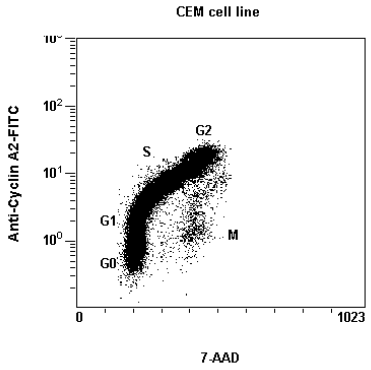
8. Keep the preparations on ice in the dark and analyze by flow cytometry within 2 hours.

EXAMPLE DATA

The histogram below is a biparametric representation, fluorescence intensity *versus* fluorescence intensity of an exponentially growing human lymphoid cell line (CEM), permeabilized according to the above protocol and stained with Anti-Cyclin A2–FITC (PN A22327) and 7-AAD Viability dye (PN IM3422). Cells in G1, S, G2 and the cyclin A2-negative mitotic (M) cells may be easily distinguished.

Acquisition is with a COULTER® EPICS® XL™ flow cytometer. Analysis is with the CXP analysis software.



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PRODUCT AVAILABILITY

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For additional information in the USA, call 800-526-7694.
Outside the USA, contact your local Beckman Coulter representative.

TRADEMARKS

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