**Product datasheet**

**Anti-Adenosine Receptor A2a antibody ab3461**

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**Overview**

**Product name**
Anti-Adenosine Receptor A2a antibody

**Description**
Rabbit polyclonal to Adenosine Receptor A2a

**Host species**
Rabbit

**Specificity**
Detects adenosine receptor A2a. This antibody does not detect other AR subtypes.

**Tested applications**
Suitable for: ICC, WB, IHC-P, IP, ICC/IF

**Species reactivity**
Reacts with: Mouse, Rat, Dog, Human

Predicted to work with: Horse

**Immunogen**
Synthetic peptide corresponding to Dog Adenosine Receptor A2a aa 373-391.
Sequence:
ESHGDMGLPDVELLSHELK

**Properties**

**Form**
Liquid

**Storage instructions**
Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C or -80°C. Avoid freeze / thaw cycle.

**Storage buffer**
Preservative: 0.05% Sodium azide
Constituents: 0.1% BSA, 99% PBS

**Purity**
Immunogen affinity purified

**Clonality**
Polyclonal

**Isotype**
IgG

**Applications**

Our Abpromise guarantee covers the use of **ab3461** in the following tested applications.
The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.
Function
Receptor for adenosine. The activity of this receptor is mediated by G proteins which activate adenylyl cyclase.

Sequence similarities
Belongs to the G-protein coupled receptor 1 family.

Domain
The cytoplasmic C-terminal domain is necessary for targeting the non-ubiquitinated form of this protein to the cell surface.

Post-translational modifications
Ubiquitinated. Deubiquitinated by USP4; leading to stabilization and expression at the cell surface.

Cellular localization
Cell membrane.

Application | Abreviews | Notes
---|---|---
ICC | 1/10 - 1/100. |  |
WB | 1/1000. |  |
IHC-P | 1/20 - 1/200. |  |
IP | Use at an assay dependent concentration. |  |
ICC/IF | Use a concentration of 10 µg/ml. |  |

Target

Function
Receptor for adenosine. The activity of this receptor is mediated by G proteins which activate adenylyl cyclase.

Sequence similarities
Belongs to the G-protein coupled receptor 1 family.

Domain
The cytoplasmic C-terminal domain is necessary for targeting the non-ubiquitinated form of this protein to the cell surface.

Post-translational modifications
Ubiquitinated. Deubiquitinated by USP4; leading to stabilization and expression at the cell surface.

Cellular localization
Cell membrane.

Images

**All lanes** : Anti-Adenosine Receptor A2a antibody (ab3461) at 1/500 dilution

**Lane 1** : Human placenta cell lysate
**Lane 2** : HepG2 cell lysate
**Lane 3** : HeLa cell lysate
**Lane 4** : Mouse liver cell lysate

Lysates/proteins at 25 µg per lane.
Immunocytochemistry/Immunofluorescence analysis of Adenosine Receptor A2a (green) showing staining in the cytoplasm of U251 cells (right) compared to a negative control (left). Formalin-fixed cells were permeabilized with 0.1% Triton X-100 in TBS for 5-10 minutes and blocked with 3% BSA-PBS for 30 minutes at room temperature. Cells were incubated with ab3461 in 3% BSA-PBS at a dilution of 1:20 overnight at 4 ºC in a humidified chamber. Cells were washed with PBST and incubated with a DyLight-conjugated secondary antibody in PBS at room temperature in the dark. F-actin (red) was stained with a fluorescent red phalloidin and nuclei (blue) were stained with Hoechst or DAPI. Images were taken at a magnification of 60x.

ab3461 labelling Adenosine Receptor A2a in the cytoplasm and membrane of Mouse testis tissue (right) compared with a negative control (left) by Immunohistochemistry (formalin/PFA-fixed paraffin-embedded sections). To expose target proteins, antigen retrieval method was performed using 10mM sodium citrate (pH 6.0) microwaved for 8-15 min. Following antigen retrieval, tissues were blocked in 3% H2O2-methanol for 15 min at room temperature. Tissue sections were incubated with the primary antibody (1:20 in 3% BSA-PBS) overnight at 4ºC. A HRP-conjugated anti-rabbit IgG was as the secondary antibody, followed by colorimetric detection using a DAB kit. Tissues were counterstained with hematoxylin and dehydrated with ethanol and xylene to prep for mounting.
ab3461 labelling Adenosine Receptor A2a in the cytoplasm and membrane of Human testis tissue (right) compared with a negative control (left) by Immunohistochemistry (formalin/PFA-fixed paraffin-embedded sections). To expose target proteins, antigen retrieval method was performed using 10mM sodium citrate (pH 6.0) microwaved for 8-15 min. Following antigen retrieval, tissues were blocked in 3% H2O2-methanol for 15 min at room temperature. Tissue sections were incubated with the primary antibody (1:200 in 3% BSA-PBS) overnight at 4°C. A HRP-conjugated anti-rabbit IgG was as the secondary antibody, followed by colorimetric detection using a DAB kit. Tissues were counterstained with hematoxylin and dehydrated with ethanol and xylene to prep for mounting.

ab3461 labelling Adenosine Receptor A2a in the cytoplasm and membrane of Human placenta tissue (right) compared with a negative control (left) by Immunohistochemistry (formalin/PFA-fixed paraffin-embedded sections). To expose target proteins, antigen retrieval method was performed using 10mM sodium citrate (pH 6.0) microwaved for 8-15 min. Following antigen retrieval, tissues were blocked in 3% H2O2-methanol for 15 min at room temperature. Tissue sections were incubated with the primary antibody (1:100 in 3% BSA-PBS) overnight at 4°C. A HRP-conjugated anti-rabbit IgG was as the secondary antibody, followed by colorimetric detection using a DAB kit. Tissues were counterstained with hematoxylin and dehydrated with ethanol and xylene to prep for mounting.
Anti-Adenosine Receptor A2a antibody (ab3461) at 1/1000 dilution + Human heart tissue lysate at 20 µg

**Secondary**
Donkey HRP-conjugate anti-rabbit at 1/5000 dilution

Developed using the ECL technique.

Performed under reducing conditions.

**Exposure time:** 3 minutes

**ICC/IF image of ab3461 stained SKNSH cells.**
The cells were 100% methanol fixed (5 min) and then incubated in 1%BSA / 10% normal goat serum / 0.3M glycine in 0.1% PBS-Tween for 1h to permeabilise the cells and block non-specific protein-protein interactions. The cells were then incubated with the antibody (ab3461, 10µg/ml) overnight at +4°C. The secondary antibody (green) was **ab96899 Dylight 488 goat anti-rabbit IgG (H+L)** used at a 1/250 dilution for 1h. **Alexa Fluor® 594 WGA** was used to label plasma membranes (red) at a 1/200 dilution for 1h. **DAPI** was used to stain the cell nuclei (blue) at a concentration of 1.43µM.

**Please note:** All products are "FOR RESEARCH USE ONLY AND ARE NOT INTENDED FOR DIAGNOSTIC OR THERAPEUTIC USE"

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