

# Product Datasheet

## Adenosine A2aR Antibody (7F6-G5-A2) - BSA Free NBP1-39474

Unit Size: 0.1 ml

Store at 4C short term. Aliquot and store at -20C long term. Avoid freeze-thaw cycles.

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**NBP1-39474**

Adenosine A2aR Antibody (7F6-G5-A2) - BSA Free

Product Information	
Unit Size	0.1 ml
Concentration	1.0 mg/ml
Storage	Store at 4C short term. Aliquot and store at -20C long term. Avoid freeze-thaw cycles.
Clonality	Monoclonal
Clone	7F6-G5-A2
Preservative	0.02% Sodium Azide
Isotype	IgG2a
Purity	Protein G purified
Buffer	PBS
Target Molecular Weight	43 kDa

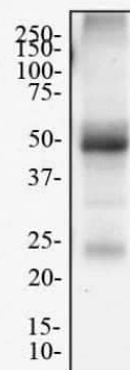
Product Description	
Description	Novus Biologicals Mouse Adenosine A2aR Antibody (7F6-G5-A2) - BSA Free (NBP1-39474) is a monoclonal antibody validated for use in IHC, WB, Flow and ICC/IF. Anti-Adenosine A2aR Antibody: Cited in 17 publications. All Novus Biologicals antibodies are covered by our 100% guarantee.
Host	Mouse
Gene ID	135
Gene Symbol	ADORA2A
Species	Human, Mouse, Rat, Porcine, Canine, Guinea Pig, Rabbit, Sheep
Reactivity Notes	Sheep reactivity reported in scientific literature (PMID: 29791204). Please note that this antibody is reactive to Mouse and derived from the same host, Mouse. Mouse-On-Mouse blocking reagent may be needed for IHC and ICC experiments to reduce high background signal. You can find these reagents under catalog numbers PK-2200-NB and MP-2400-NB. Please contact Technical Support if you have any questions.
Immunogen	Full length recombinant human Adenosine A2a R. [UniProt# P29274]

Product Application Details	
Applications	Western Blot, Immunohistochemistry-Paraffin, Flow Cytometry, Flow (Intracellular), Immunocytochemistry/ Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Frozen, CyTOF-ready
Recommended Dilutions	Western Blot 1:1000, Flow Cytometry 1-5 ug/mL, Immunohistochemistry 1 -5 ug/ml, Immunocytochemistry/ Immunofluorescence 1:50-1:100. Use reported in scientific literature (PMID 25836920), Immunohistochemistry-Paraffin 1 -5 ug/ml, Immunohistochemistry-Frozen reported in scientific literature (PMID 9822147), Flow (Intracellular) 1 - 5 ug/ml, CyTOF-ready

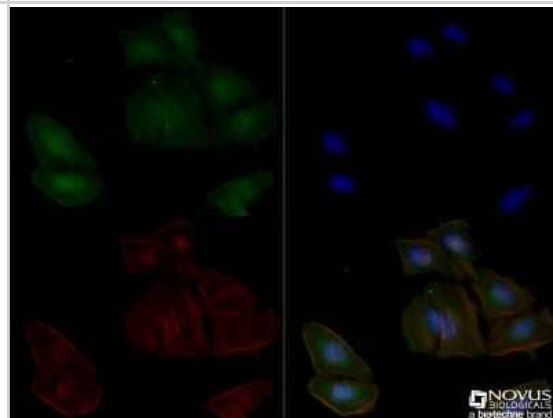


## Images

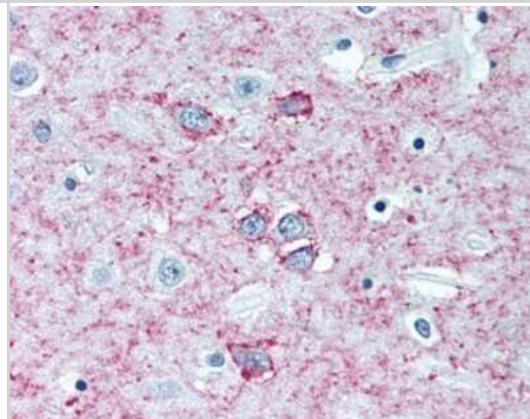
**Western Blot: Adenosine A2aR Antibody (7F6-G5-A2) [NBP1-39474]** - Total protein from mouse brain was separated on a 12% gel by SDS-PAGE, transferred to PVDF membrane and blocked in 5% non-fat milk in TBST. The membrane was probed with 1.0 ug/mL anti-Adenosine A2a R (7F6-G5-A2) in 1% milk, and detected with an anti-mouse HRP secondary antibody using chemiluminescence.



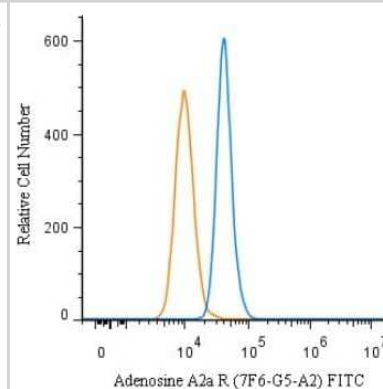
**Immunocytochemistry/Immunofluorescence: Adenosine A2aR Antibody (7F6-G5-A2) [NBP1-39474]** - HeLa cells were fixed for 10 minutes using 10% formalin and then permeabilized for 5 minutes using 1X TBS + 0.5% Triton X-100. The cells were incubated with anti-Adenosine A2a R (7F6-G5-A2), NBP1-39474, at a 1:50 dilution overnight at 4C and detected with an anti-mouse Dylight 488 (Green) at a 1:500 dilution. Actin was detected with Phalloidin 568 (Red) at a 1:200 dilution. Nuclei were counterstained with DAPI (Blue). Cells were imaged using a 40X objective.



**Immunohistochemistry-Paraffin: Adenosine A2aR Antibody (7F6-G5-A2) [NBP1-39474]** - Staining of human brain (putamen), antibody at 5 ug/mL.

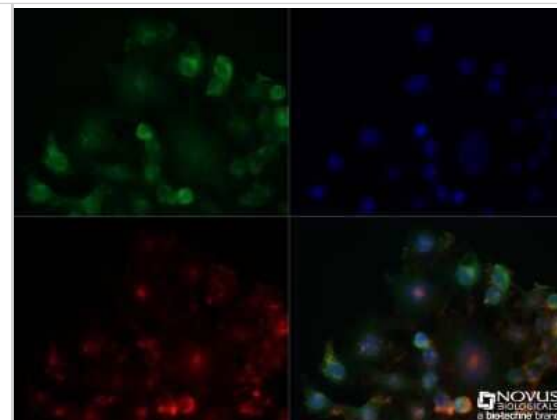


**Flow Cytometry: Adenosine A2aR Antibody (7F6-G5-A2) [NBP1-39474]** - An intracellular stain was performed on U-87 MG cells with Adenosine A2a R (7F6-G5-A2) antibody NBP1-39474F (blue) and a matched isotype control (orange). Cells were fixed with 4% PFA and then permeabilized with 0.1% saponin. Cells were incubated in an antibody dilution of 10 ug/mL for 30 minutes at room temperature. Both antibodies were conjugated to FITC.

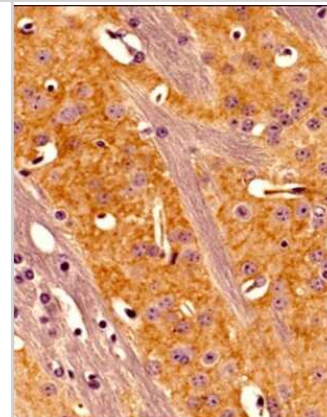


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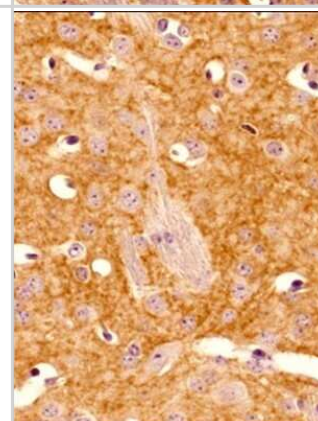
**Immunocytochemistry/Immunofluorescence: Adenosine A2aR Antibody (7F6-G5-A2) [NBP1-39474]** - Neuro2a cells were fixed for 10 minutes using 10% formalin and then permeabilized for 5 minutes using 1X TBS + 0.5% Triton X-100. The cells were incubated with anti-Adenosine A2a R (7F6-G5-A2), NBP1-39474, at a 1:50 dilution overnight at 4C and detected with an anti-mouse Dylight 488 (Green) at a 1:500 dilution. Actin was detected with Phalloidin 568 (Red) at a 1:200 dilution. Nuclei were counterstained with DAPI (Blue). Cells were imaged using a 40X objective.



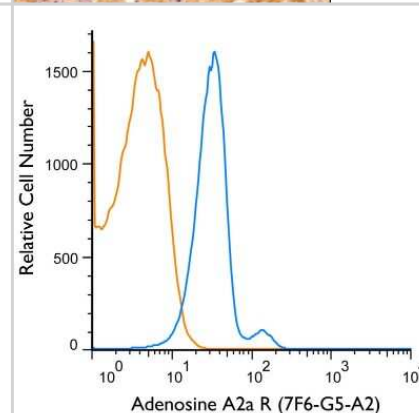
**Immunohistochemistry-Paraffin: Adenosine A2aR Antibody (7F6-G5-A2) [NBP1-39474]** - Analysis of a FFPE tissue section of mouse brain using Adenosine A2a R antibody (clone 7F6-G5-A2) at 1:100 dilution. The antibody generated nice membranous / punctate staining of Adenosine A2a receptors.



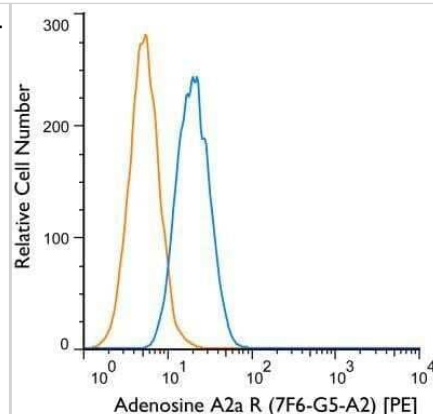
**Immunohistochemistry-Paraffin: Adenosine A2aR Antibody (7F6-G5-A2) [NBP1-39474]** - Analysis of a FFPE tissue section of mouse brain using Adenosine A2a R antibody (clone 7F6-G5-A2) at 1:400 dilution. The antibody generated nice membranous / punctate staining of Adenosine A2a receptors.



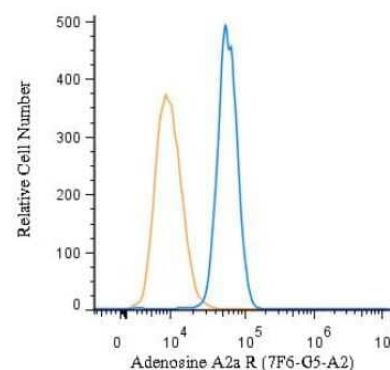
**Flow (Intracellular): Adenosine A2aR Antibody (7F6-G5-A2) [NBP1-39474]** - An intracellular stain was performed on hPBMCs with Adenosine A2a R (7F6-G5-A2) antibody NBP1-39474 and a matched isotype control NBP2-14864. Cells were fixed with 4% PFA and then permeabilized with 0.1% saponin. Cells were incubated in an antibody dilution of 1 ug/mL for 30 minutes at room temperature, followed by mouse F(ab)2 IgG (H+L) APC-conjugated secondary antibody (F0101B, R&D Systems).



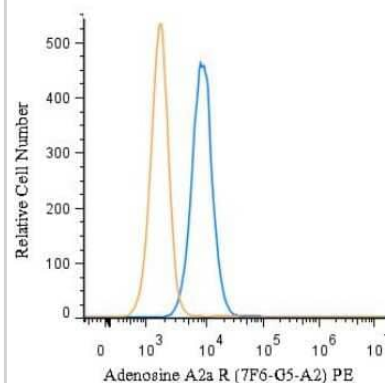
Flow Cytometry: Adenosine A2aR Antibody (7F6-G5-A2) [NBP1-39474] - Using the PE direct conjugate An intracellular stain was performed on SH-SY5Y cells with Adenosine A2a R (7F6-G5-A2) antibody NBP1-39474PE (blue) and a matched isotype control NB600-986PE (orange). Cells were fixed with 4% PFA and then permeablized with 0.1% saponin. Cells were incubated in an antibody dilution of 1 ug/mL for 30 minutes at room temperature. Both antibodies were conjugated to Phycoerythrin (PE).



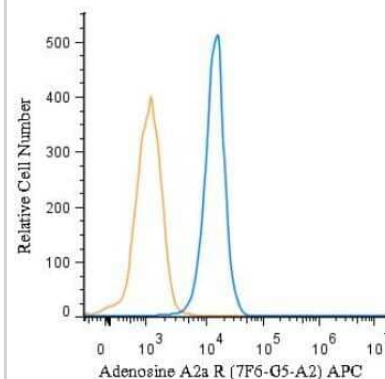
Flow (Intracellular): Adenosine A2aR Antibody (7F6-G5-A2) [NBP1-39474] - An intracellular stain was performed on U-937 cells with Adenosine A2a R (7F6-G5-A2) antibody NBP1-39474 and a matched isotype control. Cells were fixed with 4% PFA and then permeablized with 0.1% saponin. Cells were incubated in an antibody dilution of 2.5 ug/mL for 30 minutes at room temperature, followed by mouse F(ab)2 IgG (H+L) PE-conjugated secondary antibody (F0102B, R&D Systems).



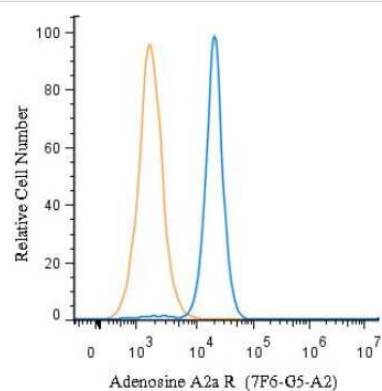
Flow (Intracellular): Adenosine A2aR Antibody (7F6-G5-A2) [NBP1-39474] - An intracellular stain was performed on U-937 cells with Adenosine A2a R (7F6-G5-A2) antibody NBP1-39474PE (blue) and a matched isotype control (orange). Cells were fixed with 4% PFA and then permeablized with 0.1% saponin. Cells were incubated in an antibody dilution of 5 ug/mL for 30 minutes at room temperature. Both antibodies were conjugated to Phycoerythrin (PE).



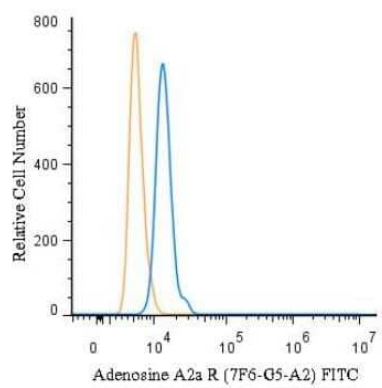
Flow (Intracellular): Adenosine A2aR Antibody (7F6-G5-A2) [NBP1-39474] - An intracellular stain was performed on U-937 cells with Adenosine A2a R (7F6-G5-A2) antibody NBP1-39474APC (blue) and a matched isotype control (orange). Cells were fixed with 4% PFA and then permeablized with 0.1% saponin. Cells were incubated in an antibody dilution of 1 ug/mL for 30 minutes at room temperature. Both antibodies were conjugated to Allophycocyanin (APC).



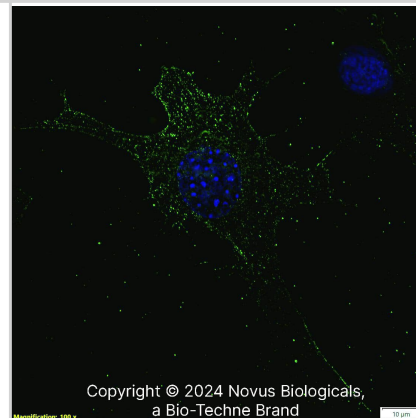
**Flow Cytometry: Adenosine A2aR Antibody (7F6-G5-A2) [NBP1-39474] -**  
 An intracellular stain was performed on SH-SY5Y with Adenosine A2a R Antibody (7F6-G5-A2) NBP1-39474 and a matched isotype control. Cells were fixed with 4% PFA and then permeabilized with 0.1% saponin. Cells were incubated in an antibody dilution of 1 ug/mL for 30 minutes at room temperature, followed by Mouse F(ab)2 IgG (H+L) PE-conjugated Antibody (R&D Systems, F0102B).



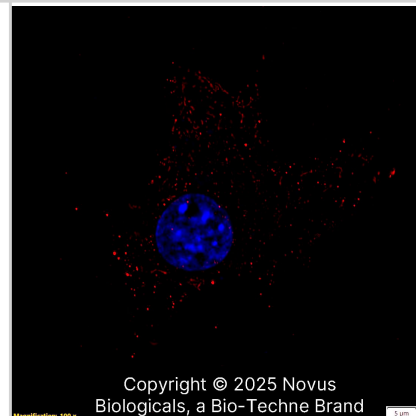
**Flow Cytometry: Adenosine A2aR Antibody (7F6-G5-A2) [NBP1-39474] -**  
 An intracellular stain was performed on U-937 cells with Adenosine A2a R (7F6-G5-A2) antibody NBP1-39474F (blue) and a matched isotype control (orange). Cells were fixed with 4% PFA and then permeabilized with 0.1% saponin. Cells were incubated in an antibody dilution of 5 ug/mL for 30 minutes at room temperature. Both antibodies were conjugated to FITC.



Adenosine A2aR was detected in immersion fixed NIH3T3 Mouse fibroblast cell line using Anti- Mouse anti-Adenosine A2aR (7F6-G5-A2) Protein-G purified Monoclonal Antibody (Catalog # NBP1-39474) at 1.0 ug/mL overnight at 4C. Cells were stained using DyLight 488-conjugated Anti-Mouse IgG (H+L) Cross-Absorbed Secondary Antibody (green) and counterstained with DAPI (blue). Cells were imaged using a 100X objective and digitally deconvolved.



Adenosine A2aR was detected in immersion fixed NIH3T3 Mouse fibroblast cell line using Anti- Mouse anti-Adenosine A2aR (7F6-G5-A2) Protein-G purified Monoclonal Antibody conjugated to Biotin (Catalog # NBP1-39474B) at 5 µg/mL overnight at 4C. Cells were stained using Streptavidin conjugated to DyLight 550 (red) and counterstained with DAPI (blue). Cells were imaged using a 100X objective and digitally deconvolved.



## Publications

Allard B, Jacobberger-Foissac C, Cousineau I et al. Adenosine A2A receptor is a tumor suppressor of NASH-associated hepatocellular carcinoma Cell Rep Med 2023-09-19 [PMID: 37729873] (Western Blot, Immunohistochemistry-Frozen, Immunocytochemistry, Rat)

Ott M, Tomaszowski KH, Marisetty A, Kong LY et al. Profiling of patients with glioma reveals the dominant immunosuppressive axis is refractory to immune function restoration JCI Insight 2020-07-30 [PMID: 32721947]

Tokano M, Matsushita S, Takagi R Et al. Extracellular adenosine induces hypersecretion of IL-17A by T-helper 17 cells through the adenosine A2a receptor Brain Behav Immun Health 2022-12-05 [PMID: 36467126] (FLOW, Mouse)

### Details:

Citation using the PE version of this antibody.

Nguyen KD. Inflammatory Cytokines Regulate Adenosine A2A Receptor Expression, Function, and Desensitization. Int J Interferon Cytokine Mediat Res 2010-12-07 [PMID: 21132069]

Basu, M, Gupta, P Et al. Increased host ATP efflux and its conversion to extracellular adenosine is crucial for establishing Leishmania infection. J Cell Sci 2020-04-08 [PMID: 32079656] (WB, Human)

Baum N, Fliegert R, Bauche A, et al. Daratumumab and Nanobody-Based Heavy Chain Antibodies Inhibit the ADPR Cyclase but not the NAD<sup>+</sup> Hydrolase Activity of CD38-Expressing Multiple Myeloma Cells Cancers 2020-12-30 [PMID: 33396591] (Human)

Hirata Y, Furuhashi K, Ishii H et al. CD150(high) Bone Marrow Tregs Maintain Hematopoietic Stem Cell Quiescence and Immune Privilege via Adenosine Cell Stem Cell. 2018-03-01 [PMID: 29456159]

### Details:

Citation using the Alexa Fluor 647 form of this antibody.

Chimote AA, Balajthy A, Arnold MJ, et al. A defect in KCa3.1 channel activity limits the ability of CD8<sup>+</sup> T cells from cancer patients to infiltrate an adenosine-rich microenvironment. Sci Signal. 2018-04-24 [PMID: 29692361] (FLOW, Human)

Davis L, Musso J, Soman D et al. ROLE OF ADENOSINE SIGNALING IN COORDINATING CARDIOMYOCYTE FUNCTION AND CORONARY VASCULAR GROWTH IN CHRONIC FETAL ANEMIA Am. J. Physiol. Regul. Integr. Comp. Physiol. 2018-05-23 [PMID: 29791204] (IF/IHC, Sheep)

Mediavilla-Varela M, Castro J, Chiappori A et al. A Novel Antagonist of the Immune Checkpoint Protein Adenosine A2a Receptor Restores Tumor-Infiltrating Lymphocyte Activity in the Context of the Tumor Microenvironment. Neoplasia. 2017-06-02 [PMID: 28582704] (FLOW, Human)

Haschemi A, Wagner O, Marculescu R et al. Cross-regulation of carbon monoxide and the adenosine A2a receptor in macrophages. J Immunol. 2007-05-01 [PMID: 17442976] (WB, ICC/IF, Mouse)

Nguyen DK, Montesinos MC, Williams AJ et al. Th1 cytokines regulate adenosine receptors and their downstream signaling elements in human microvascular endothelial cells. J Immunol. 2003-10-15 [PMID: 14530318] (WB, Human)

### Details:

Adenosine A2a R antibody (clone 7F6-G5-A2) was used for Western blot analysis of adenosine A2A receptors in Human Microvascular Endothelial Cells (HMVECs) which were treated overnight with IL-1, TNF-alpha, or IFN-gamma.

More publications at <http://www.novusbio.com/NBP1-39474>

## Procedures

### Western Blot Protocol for Adenosine A2aR Antibody (NBP1-39474)

#### Western Blot Protocol

1. Perform SDS-PAGE on samples to be analyzed, loading 10-25 ug of total protein per lane.
2. Transfer proteins to PVDF membrane according to the instructions provided by the manufacturer of the membrane and transfer apparatus.
3. Stain the membrane with Ponceau S (or similar product) to assess transfer success, and mark molecular weight standards where appropriate.
4. Rinse the blot TBS -0.05% Tween 20 (TBST).
5. Block the membrane in 5% Non-fat milk in TBST (blocking buffer) for at least 1 hour.
6. Wash the membrane in TBST three times for 10 minutes each.
7. Dilute primary antibody in blocking buffer and incubate overnight at 4C with gentle rocking.
8. Wash the membrane in TBST three times for 10 minutes each.
9. Incubate the membrane in diluted HRP conjugated secondary antibody in blocking buffer (as per manufacturer's instructions) for 1 hour at room temperature.
10. Wash the blot in TBST three times for 10 minutes each (this step can be repeated as required to reduce background).
11. Apply the detection reagent of choice in accordance with the manufacturers instructi

### Immunocytochemistry/Immunofluorescence Protocol for Adenosine A2aR Antibody (NBP1-39474)

#### Immunocytochemistry Protocol

Culture cells to appropriate density in 35 mm culture dishes or 6-well plates.

1. Remove culture medium and wash the cells briefly in PBS. Add 10% formalin to the dish and fix at room temperature for 10 minutes.
2. Remove the formalin and wash the cells in PBS.
3. Permeablize the cells with 0.1% Triton X100 or other suitable detergent for 10 min.
4. Remove the permeablization buffer and wash three times for 10 minutes each in PBS. Be sure to not let the specimen dry out.
5. To block nonspecific antibody binding, incubate in 10% normal goat serum from 1 hour to overnight at room temperature.
6. Add primary antibody at appropriate dilution and incubate overnight at 4C.
7. Remove primary antibody and replace with PBS. Wash three times for 10 minutes each.
8. Add secondary antibody at appropriate dilution. Incubate for 1 hour at room temperature.
9. Remove secondary antibody and replace with PBS. Wash three times for 10 minutes each.
10. Counter stain DNA with DAPI if required.



**Immunohistochemistry-Paraffin Protocol for Adenosine A2aR Antibody (NBP1-39474)**

## Immunohistochemistry-Paraffin Embedded Sections

## Antigen Unmasking:

Bring slides to a boil in 10 mM sodium citrate buffer (pH 6.0) then maintain at a sub-boiling temperature for 10 minutes. Cool slides on bench-top for 30 minutes (keep slides in the sodium citrate buffer all the time).

## Staining:

1. Wash sections in deionized water three times for 5 minutes each.
2. Wash sections in PBS for 5 minutes.
3. Block each section with 100-400 ul blocking solution (1% BSA in PBS) for 1 hour at room temperature.
4. Remove blocking solution and add 100-400 ul diluted primary antibody. Incubate overnight at 4 C.
5. Remove antibody solution and wash sections in wash buffer three times for 5 minutes each.
6. Add 100-400 ul HRP polymer conjugated secondary antibody. Incubate 30 minutes at room temperature.
7. Wash sections three times in wash buffer for 5 minutes each.
8. Add 100-400 ul DAB substrate to each section and monitor staining closely.
9. As soon as the sections develop, immerse slides in deionized water.
10. Counterstain sections in hematoxylin.
11. Wash sections in deionized water two times for 5 minutes each.
12. Dehydrate sections.
13. Mount coverslips.



**Flow (Intracellular) Protocol for Adenosine A2aR Antibody (NBP1-39474)**

## Protocol for Flow Cytometry Intracellular Staining

## Sample Preparation.

1. Grow cells to 60-85% confluency. Flow cytometry requires between  $2 \times 10^5$  and  $1 \times 10^6$  cells for optimal performance.
2. If cells are adherent, harvest gently by washing once with staining buffer and then scraping. Avoid using trypsin as this can disrupt certain epitopes of interest. If enzymatic harvest is required, use Accutase, Collagenase, or TrypLE Express for a less damaging option.
3. Reserve 100  $\mu$ L for counting, then transfer cell volume into a 50 mL conical tube and centrifuge for 8 minutes at 400 RCF.
  - a. Count cells using a hemocytometer and a 1:1 trypan blue exclusion stain to determine cell viability before starting the flow protocol. If cells appear blue, do not proceed.
4. Re-suspend cells to a concentration of  $1 \times 10^6$  cells/mL in staining buffer (NBP2-26247).
5. Aliquot out 100  $\mu$ L samples in accordance with your experimental samples.

Tip: When cell surface and intracellular staining are required in the same sample, it is advisable that the cell surface staining be performed first since the fixation and permeabilization steps might reduce the availability of surface antigens.

## Intracellular Staining.

Tip: When performing intracellular staining, it is important to use appropriate fixation and permeabilization reagents based upon the target and its subcellular location. Generally, our Intracellular Flow Assay Kit (NBP2-29450) is a good place to start as it contains an optimized combination of reagents for intracellular staining as well as an inhibitor of intracellular protein transport (necessary if staining secreted proteins). Certain targets may require more gentle or transient permeabilization protocols such as the commonly employed methanol or saponin-based methods.

## Protocol for Cytoplasmic Targets:

1. Fix the cells by adding 100  $\mu$ L fixation solution (such as 4% PFA) to each sample for 10-15 minutes.
2. Permeabilize cells by adding 100  $\mu$ L of a permeabilization buffer to every  $1 \times 10^6$  cells present in the sample. Mix well and incubate at room temperature for 15 minutes.
  - a. For cytoplasmic targets, use a gentle permeabilization solution such as 1X PBS + 0.5% Saponin or 1X PBS + 0.5% Tween-20.
  - b. To maintain the permeabilized state throughout your experiment, use staining buffer + 0.1% of the permeabilization reagent (i.e. 0.1% Tween-20 or 0.1% Saponin).
3. Following the 15 minute incubation, add 2 mL of the staining buffer + 0.1% permeabilizer to each sample.
4. Centrifuge for 1 minute at 400 RCF.
5. Discard supernatant and re-suspend in 100  $\mu$ L of staining buffer + 0.1% permeabilizer.
6. Add appropriate amount of each antibody (eg. 1 test or 1  $\mu$ g per sample, as experimentally determined).
7. Mix well and incubate at room temperature for 30 minutes- 1 hour. Gently mix samples every 10-15 minutes.
8. Following the primary/conjugate incubation, add 1-2 mL/sample of staining buffer + 0.1% permeabilizer and centrifuge for 1 minute at 400 RCF.
9. Wash twice by re-suspending cells in staining buffer (2 mL for tubes or 200  $\mu$ L for wells) and centrifuging at 400 RCF for 5 minutes. Discard supernatant.
10. Add appropriate amount of secondary antibody (as experimentally determined) to each sample.
11. Incubate at room temperature in dark for 20 minutes.
12. Add 1-2 mL of staining buffer and centrifuge at 400 RCF for 1 minute and discard supernatant.
13. Wash twice by re-suspending cells in staining buffer (2 mL for tubes or 200  $\mu$ L for wells) and centrifuging at 400 RCF for 5 minutes. Discard supernatant.
14. Resuspend in an appropriate volume of staining buffer (usually 500  $\mu$ L per sample) and proceed with analysis on your flow cytometer.



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Orders: nb-customerservice@bio-techne.com  
General: novus@novusbio.com

### **Products Related to NBP1-39474**

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NBP2-33376H	Blue Marker Antibody (6F4-F6) [HRP]
HAF007	Goat anti-Mouse IgG Secondary Antibody [HRP]
NB7539	Goat anti-Mouse IgG (H+L) Secondary Antibody [HRP]
NBP1-96778	Mouse IgG2a Isotype Control (M2A)

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### **Limitations**

This product is for research use only and is not approved for use in humans or in clinical diagnosis. Primary Antibodies are guaranteed for 1 year from date of receipt.

For more information on our 100% guarantee, please visit [www.novusbio.com/guarantee](http://www.novusbio.com/guarantee)

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