

DATASHEET

LUF7960

Product overview

Name LUF7960
Cat No HB8396
Biological description Novel, adenosine A₃AR Affinity-Based Probe (AfBP) which is suitable for click conjugation for use in confocal microscopy, SDS-PAGE and detection of endogenous hA₃AR in flow cytometry.

Binds covalently to the hA₃AR (apparent pK_i values at A₁AR are 7.27 and 8.4 (following a 4h preincubation))

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Biological action Agonist
Purity >98%
Description Novel, clickable Adenosine hA₃AR Affinity-Based Probe (AfBP).

Images

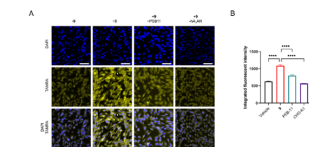
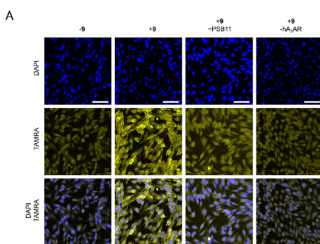


Figure 4. Labeling of the hA₃AR observed by confocal microscopy. CHO cells with (CHO+hA₃AR) or without (CHO-K1) stable expression of the hA₃AR were pre-incubated for 4h with PBS (1 μM final concentration) or 10 (PSB11) antagonist and incubated for 40 min with or without TAMRA (click reagent). Cells were fixed, permeabilized, and subjected to a copper-catalyzed click reaction with TAMRA (1 μM final concentration). The cells were then washed and kept in PBS containing 200 μM DAPI during confocal imaging. (A) Shows an image showing blue nuclei, TAMRA staining (green), merged image, and magnified view of each image (top row). Images were acquired automatically at a distance of 10 μm in the x-y plane and an acquisition time less than 1 minute per image. Scale bar: 10 μm. (B) Shows an image showing TAMRA signal (green) in the cell. Comparison of the integrated fluorescence intensity between treatment conditions. Data were obtained from 2 x 4 fields of view. Three color representations performed in duplicate. Each data point represents the integrated fluorescence intensity of the TAMRA signal per individual cell. Shown as the bar graphs is the average integrated fluorescence intensity of all individual cells. Error bars represent SEM. Significance was calculated using a one-way ANOVA test using multiple comparisons. A significant increase in intensity is observed for the cells containing the hA₃AR and treated with N₃ versus the other conditions.

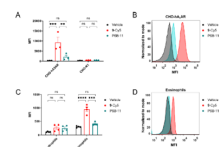
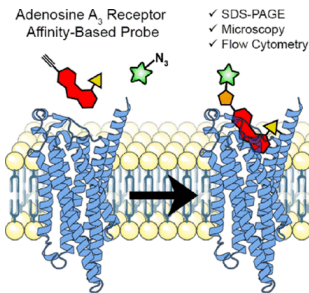


Figure 5. Labeling of the hA₃AR in flow cytometry experiments. Samples were pre-incubated for 4h with the antagonist PSB11 and incubated for 40 min with or without 1 μM N₃ (agonist) or 10 μM antagonist (PSB11). Samples were then washed and subjected to a copper-catalyzed click reaction with TAMRA. (A) SDS-PAGE analysis of hA₃AR labeling. (B) Flow cytometry analysis of hA₃AR labeling. (C) SDS-PAGE analysis of hA₃AR labeling. (D) Flow cytometry analysis of hA₃AR labeling. Values represent the integrated fluorescence intensity of all individual cells. Error bars represent SEM. Significance was calculated by a one-way ANOVA test using multiple comparisons (**p < 0.01; ***p < 0.001; ns = not significant). (E) Representative graph showing the observed shift in MFI related to hA₃AR labeling in CHO+hA₃AR cells. (F) MFI of CHO-K1 untreated and untreated parallel from CHO+hA₃AR cells. Values represent the integrated fluorescence intensity of all individual cells. Error bars represent SEM. Significance was calculated by a one-way ANOVA test using multiple comparisons (**p < 0.01; ***p < 0.001; ns = not significant). (G) Representative graph showing the observed shift in MFI related to hA₃AR labeling in human neuroblastoma.

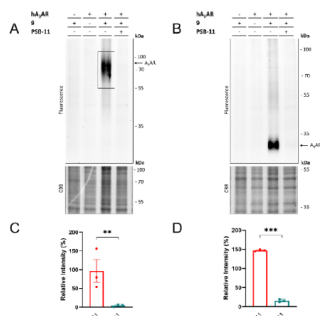


Figure 5. Labeling of the hA₃AR on live CHO cells. CHO cells with or without (first lane) stable expression of the hA₃AR were pre-incubated for 1 h with antagonist (PSB-11, 1 μM final concentration) at 37 °C, prior to incubation with 9 (50 nM final concentration) for 1 h at 37 °C. After the incubation, the unbound probe was washed away with PBS. Membranes were prepared, brought to a concentration of 1 μg/μL, and subjected to the copper-catalyzed click reaction with Cy5-N₃ (1 μM final concentration). Samples were then denatured with Laemmli buffer (4x), resolved by SDS-PAGE, and imaged using gel fluorescence. Gels were stained by Coomassie Brilliant Blue (CBB) as loading control. (A) Labeling of glycosylated hA₃AR. (B) Labeling of deglycosylated hA₃AR. PNGase was added prior to the addition of click reagents. (C, D) Quantification of the observed signals with and without addition of antagonist (PSB-11). The band intensities were calculated using ImageLab and corrected for the amount of protein measured after CBB staining. The band at 55 kDa of the PageRuler Plus ladder (not shown) was set to 100% for each gel and band intensities were calculated relative to this band. The mean values ± SEM of three individual experiments are shown. Significance was calculated by a two-way ANOVA test using multiple comparisons (***p < 0.001; **p < 0.01).

Solubility & Handling

Storage instructions
Important

-20 °C

This product is for RESEARCH USE ONLY and is not intended for therapeutic or diagnostic use. Not for human or veterinary use.

Chemical Data

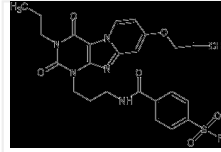
Chemical name

4-[3-(2,4-Dioxo-3-propyl-8-prop-2-ynoxypurino[7,8-a]pyridin-1-yl)propylcarbamoyl]benzenesulfonyl fluoride

Molecular Weight

541.55

Chemical structure



Molecular Formula

C₂₅H₂₄FN₅O₆S

PubChem identifier

168510594

SMILES

CCCN1C(=O)C2=C(N=C3N2C=CC(=C3)OCC#C)N(C1=O)CCCNC(=O)C4=CC=C(C=C4)S(=O)(=O)F

InChiKey

GQJPGXUBGVKCMG-UHFFFAOYSA-N

Licensing details

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References

Development of an Affinity-Based Probe to Profile Endogenous Human Adenosine A(3) Receptor Expression.

Beerkens BLH et al (2023) Journal of medicinal chemistry 66

PubMedID

[37531576](#)
