

DATASHEET

LUF7960

Product overview

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| Name | LUF7960 |
| Cat No | HB8396 |
| Biological description | Novel, adenosine A ₃ AR Affinity-Based Probe (AfBP) which is suitable for click conjugation for use in confocal microscopy, SDS-PAGE and detection of endogenous hA ₃ AR in flow cytometry. Binds covalently to the hA ₃ AR (apparent pK _i values at A ₁ AR are 7.27 and 8.4 (following a 4h preincubation) |
| <i>Sold under license from the Oncode Institute and Universiteit Leiden</i> | |
| Biological action | Agonist |
| Purity | >98% |
| Description | Novel, clickable Adenosine hA ₃ AR Affinity-Based Probe (AfBP). |

Images

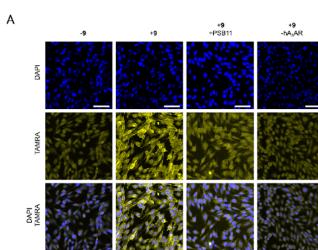


Figure 6. Labeling of the hAAR observed by confocal microscopy. CHO cells with (CHO-hAAR) or without (CHO-K1) stable expression of the hAAR were pre-incubated for 30 min with PBS-11 (1 μ M final concentration) or 1% DMSO (control) and incubated for 60 min with 90 nM biotin (blue, first wash), 100 nM streptavidin-allophycocyanin (red, second wash), and 100 nM of either biotin (blue) or streptavidin (red) (third wash). The cells were then washed and kept in PBS containing 300 mM DMSO during confocal imaging. (A) Shows DAPI staining (blue, first wash), TMAA staining (yellow, second wash), and a merge of both stains (third wash). Images were acquired automatically at 1024 \times 1024 pixels. (B) Comparison of the integrated fluorescence intensity between treatment conditions. Data was obtained from 2 \times 6 fields of view, from the same experiment as in (A). The error bars represent the standard deviation of the mean. The asterisk indicates a significant increase in intensity in the bar graph is the average integrated fluorescence intensity of all individual cells \pm SEM. Significance was calculated using a one-way ANOVA, followed by a Tukey's HSD test. A significant increase in intensity is observed in the cells containing the hAAR and treated with 90 nM biotin under conditions.

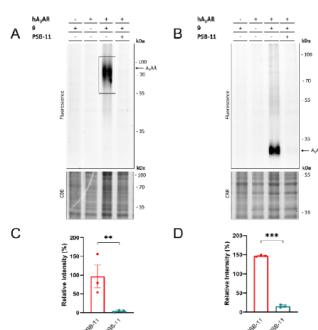


Figure 5. Labeling of the h₃AR on live CHO cells. CHO cells with or without (first lane) stable expression of the h₃ARs were preincubated for 1 h with antagonist (PSB-11, 1 μ M final concentration) at 37 °C, prior to incubation with 9 (50 nM final concentration) for 1 h at 37 °C. After the incubation, the unbound probe was washed away with PBS. Membranes were prepared, brought to a concentration of 1 μ g/ μ L, and subjected to the copper-catalyzed click reaction with Cy5-N₃ (1 μ M final concentration). Samples were then denatured with Laemmli buffer (4x), resolved by SDS-PAGE, and visualized using in gel fluorescence. Gels were stained by Coomassie Brilliant Blue (CBB) as loading control. (A) Labeling of glycosylated h₃AR. (B) Labeling of deglycosylated h₃AR. 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 \pm SEM of three individual experiments are shown. Significance was calculated by a two-way ANOVA test using multiple comparisons (** p < 0.01; * p < 0.05).

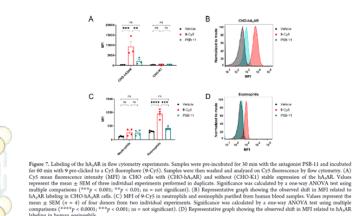
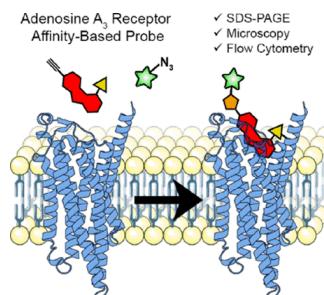


Figure 7. Labeling of the λ -NLR in five cyanophytes. Samples were pre-inoculated with λ and incubated for 60 min with λ pre-locked to a Cys55 antibody (Cys55). Samples were then washed and analyzed on a Cy5 fluorescence by flow cytometry. (A) Cys55 mean ± SEM of three independent experiments. (B) Representative graph showing the observed shift in MFI related to multiple comparisons ($^{***}p < 0.001$, $^{**}p < 0.01$, $^{*}p < 0.05$ or $n = 1$ not significant). (C) Representative graph showing the observed shift in MFI related to multiple comparisons ($^{***}p < 0.001$, $^{**}p < 0.01$, $^{*}p < 0.05$ or $n = 1$ not significant). (D) Representative graph showing the observed shift in MFI related to multiple comparisons ($^{***}p < 0.001$, $^{**}p < 0.01$, $^{*}p < 0.05$ or $n = 1$ not significant). (E) Representative graph showing the observed shift in MFI related to multiple comparisons ($^{***}p < 0.001$, $^{**}p < 0.01$, $^{*}p < 0.05$ or $n = 1$ not significant).

Solubility & Handling

Storage instructions -20°C
Important This product is for RESEARCH USE ONLY and is not intended for therapeutic or diagnostic use. Not for human or veterinary use.

Chemical Data

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| 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 | C ₂₅ H ₂₄ FN ₅ O ₆ S |
| 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 | Sold under license from the Oncode Cancer Institute and Universiteit Leiden |

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