funakoshi
FRONTIERS IN LIFE SCIENCE

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LiveReceptor $GABA_AR$ <$GABA_AR$ Labeling Reagent>

Catalog NO. FDV-0018B

Product Background

Neurotransmitter receptors including glutamate receptors and GABA receptors etc. located on post-synapse in neuronal cells play various roles in brain functions. To understand physiological roles of neurotransmitter receptors, live cell imaging is one of the powerful approaches. Conventional imaging methods on live cells relay on a genetically engineered proteins fused with fluorescent proteins such as GFP. However, one serious problem is that the functions and movement of over-expressed neurotransmitter receptors with non-physiological tags are not precisely correlated with endogenous native receptors. The labeling methods for endogenous receptors are desirable to observe physiological functions of receptors.

LiveReceptor is the world first reagent series for target-specific receptor labeling. The principle of LiveReceptor is based on **l**igand-**d**irected **a**cyl **i**midazole (LDAI) chemistry (ref.1,2). LDAI-based chemical labelling is driven by selective ligand-protein recognition, which facilitates an acyl substitution reaction of labeling reagents on nucleophilic amino acid residues including Lys, Ser and Tyr located near ligand-binding domain. After wash out, the labelled receptors which have free ligand-binding pockets are observed on live cells. Furthermore, based on pH-dependent fluorescent property of fluorescein, fluorescent signal of labeled receptors in endocytosis pathway are highly quenched and only cell surface receptors can be observed. LiveReceptors are powerful tools to monitor reduction of cell surface receptors by endocytosis upon extracellular stimulation.

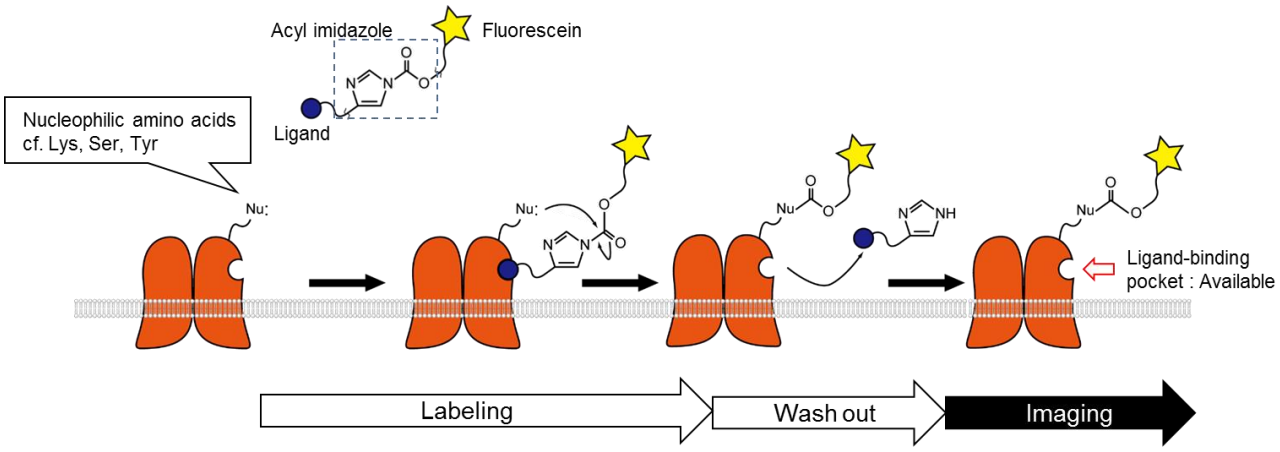


Figure 1. Principle of ligand-directed protein labeling

"LiveReceptor $GABA_AR$" is a specific labeling reagent for cell-surface ion chanel-type GABA receptor ($GABA_AR$) which is the key component for inhibitory synaptic regulation (ref.3). LiveReceptor $GABA_AR$ has three domains including gabazine as an affinity ligand for $GABA_AR$, fluorescein and acyl imidazole. Only when gabazine binds to $GABA_ARs$, nucleophilic amino acid residues (Lys, Ser or Tyr) located near ligand-binding domain on $GABA_ARs$ are attacked acyl imidazole and fluorescein is transferred into $GABA_ARs$. After removing excess reagents and resultant ligand moiety, labeled $GABA_ARs$ can be observed in both live and fixed cells. The protocol is very simple, no genetic manipulation and additional treatment are required. Because LiveReceptor $GABA_ARs$ shows no cell membrane permeability, only cell surface $GABA_ARs$ are labelled. Ref.3 indicates fluorescein-labeled $GABA_ARs$ by LiveReceptor has little effects on its ion channel capability.

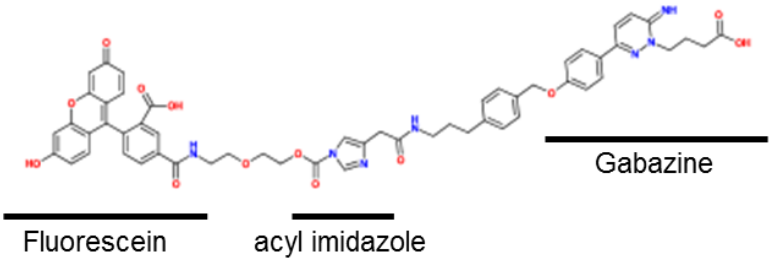


Figure 2. Chemical structure of LiveReceptor $GABA_AR$

Description

Catalog Number: FDV-0018B
Size : 10 µg
Formulation : $C_{55}H_{51}N_7O_{13}$
Molecular weight : 1017.35 g/mol
Visibility : Orange lyophilized powder
Solubility : Soluble in DMSO
*This compound has water-solubility but it can be easily degraded in water and culture medium. Please avoid store in the water.

Spectrum

Excitation/ Emission: 495/515 nm
*Compatible with FITC filter

Application

- Live cell imaging
- Immunocytochemistry with specific antibodies
- Immunoprecipitation with anti-fluorescein antibody
- Immunoblotting with anti-fluorescein antibody
- Drug screening for competitive $GABA_AR$ antagonist (GABA-site)

Reconstitution and Storage

Reconstitution :

Reconstitute at 0.1 mM (x100) - 1 mM (x1000) in 100% DMSO. Please optimize the final concentration of DMSO depended on your experiments. Before reconstitution, please spin down to collect the orange lyophilized powder on the bottom of a tube. Carefully add DMSO into the tube and vigorously mix to completely dissolve the powder.

Storage:

(powder) Store at -20ºC. Protected from light.

(solution) DMSO stock solution is stable at least for 1 year at -80ºC. Please make aliquots and avoid freeze and throw. Protected from light.

How to use

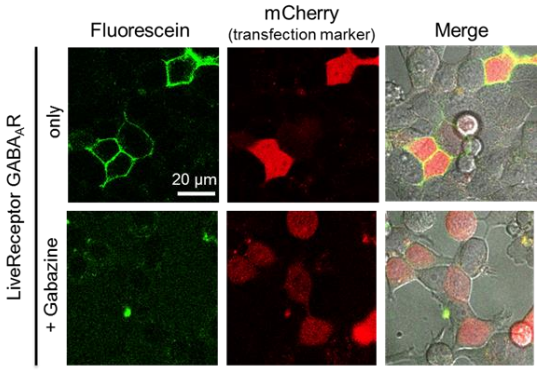
General procedure for $GABA_AR$ labeling

1. Prepare 1 μM of LiveReceptor $GABA_AR$ in the appropriate medium
   * Note : Serum-free media are highly recommended. This compound is not stable in the medium. Please prepare assay solution at time of use.
2. Replace media of cultured cells to LiveReceptor containing medium.
3. Culture cells with LiveReceptor $GABA_AR$ for 1-4 hours at 17-37ºC
4. After labeling, wash cells several times or perfused continuously to remove excess reagents.
5. Labelled $GABA_AR$s can be observed.

Application data

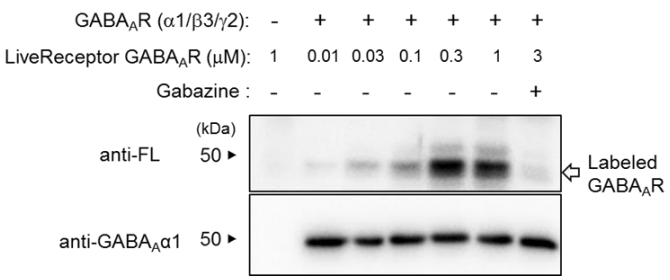
Live cell imaging of labelled $GABA_AR$s in $GABA_AR$-expressed HEK293

$GABA_AR$ (α1/β3/γ2)-expressed HEK293 cells were treated with 1 μM of LiveReceptor $GABA_AR$ in the absence or presence of 100 μM gabazine, a $GABA_AR$ selective inhibitor, for 3 hour at 37ºC and washed out three times with the basal medium. (scale bars, 20 μm)



Validation of $GABA_AR$ labeling by western blotting

$GABA_AR$ (α1/β3/γ2)-expressed HEK293 cells were treated with 0.01-1 μM of LiveReceptor $GABA_AR$ in the absence or presence of 100 μM gabazine, a $GABA_AR$ selective inhibitor, for 3 hour at 37ºC. After wash cells, the cells were lysed and proteins were applied into SDS-PAGE and western blotting using anti-fluorescein (FL) antibody or anti-$GABA_AR$ α1 subunit.



Reference

1. Fujishima *et al.*, *J. Am. Chem. Soc.*, **134**, 3961-3964 (2012). Ligand-directed acyl imidazole chemistry for labeling of membrane-bound proteins on live cells.
2. Miki *et al.*, *Chem. Biol.*, **21**, 1013-1022 (2014). LDAI-based Chemical Labeling of Intact Membrane Proteins and its Pulse-Chase Analysis under Live Cell Conditions.
3. Yamaura *et al.*, *Nat. Chem. Biol.*, **12**, 822-830 (2016). Discovery of allosteric modulators for $GABA_A$ receptors by ligand-directed chemistry.

Related product

LiveReceptor AMPAR <Endogenous AMPAR Labeling Reagent>

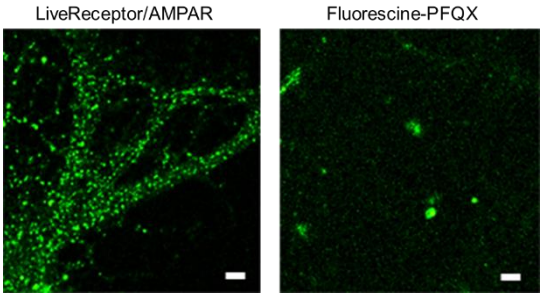
LiveReceptor AMPAR is a specific labeling reagent for AMPA-type glutamate receptor, AMPAR. Live imaging of cultured neuron and slice tissues were validated.

Catalog No. FDV-0018A
Size 10 μg

Data examples

- Live cell imaging of labelled endogenous AMPARs in cultured neurons

Cultured hippocampal neurons were treated with 1 μM of LiveReceptor AMPAR (in left) or Fluorescein-conjugated PFQX as negative control (in right) for 1 hour at 17°C and washed out three times with the basal medium. Dendritic spin-like punctual structures were observed on live cells by specifically LiveReceptor. (scal bars, 10 μm)

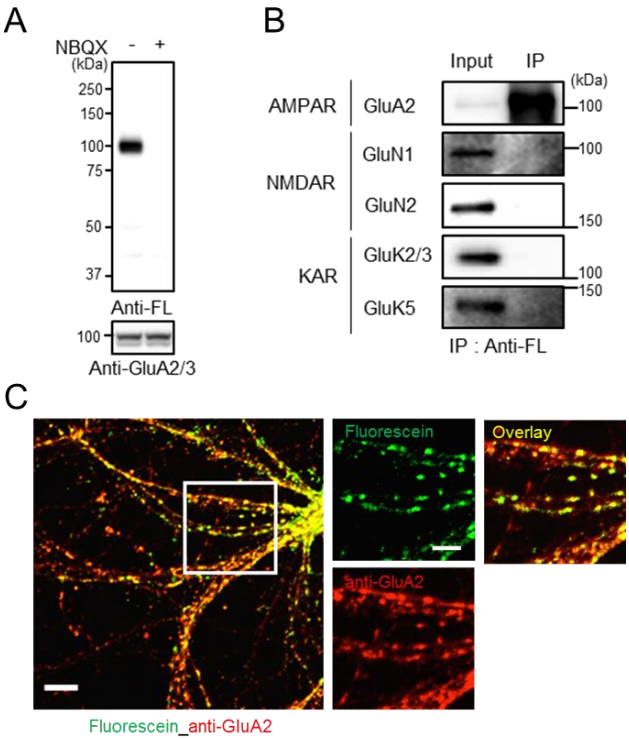


- Validation of specificity for AMPARs

A. Hippocampal slices were treated with 1 μM of LiveReceptor AMPAR in the absence or presence of 10 μM NBQX. The cell lysates were analyzed by western blotting using anti-fluorescein or anti-GluA2/3 antibody. A single band was observed by anti-fluorescein antibody and this band was dramatically disappeared by NBQX.

B. Cultured cortical neurons were treated with 1 μM of LiveReceptor AMPAR. After lysis of cultured neurons, the cell lysate was immunoprecipitated with anti-fluorescein antibody. The immunoprecipitates were analyzed by western blot using glutamate receptor-specific antibodies including GluA2 (AMPAR), GluN1 and GluN2 (NMDAR) and GluK2/3 and GluK5 (KAR). Only GluA2 was concentrated by anti-fluorescein (FL) antibody.

C. Cultured hippocampal neurons labelled with 1 μM of LiveReceptor AMPAR were fixed, permeabilized and immunostained using anti-GluA2 antibody. Fluorescein signals were well corresponding with the signal of anti-GluA2 antibodies. (Scale bar, 10 mm and 5 mm)



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