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FRONTIERS IN LIFE SCIENCE

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LiveReceptor AMPAR <Endogenous AMPAR Labeling Reagent>

Catalog No. FDV-0018A

Research use only, not for human or animal therapeutic or diagnostic use.

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.

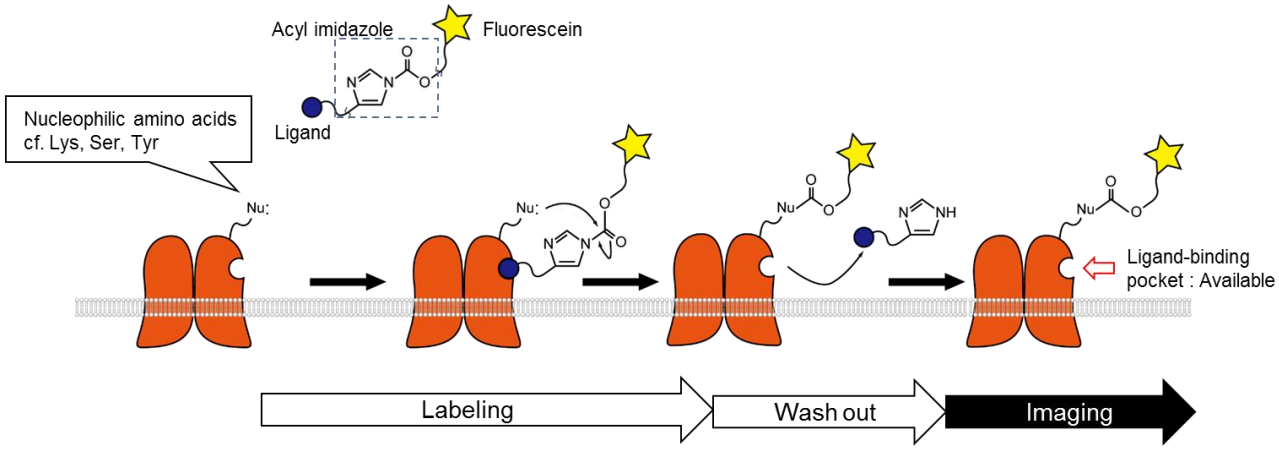


Figure 1. Principle of ligand-directed protein labeling

"LiveReceptor AMPAR" is a specific labeling reagent for cell-surface AMPA-type glutamate receptors (AMPARs) which is the key component for synaptic plasticity related to memory and learning (ref.3). LiveReceptor AMPAR has three domains including PFQX (6-pyrrolyl-7-trifluoromethyl-quinoxaline-2,3-dione) as an affinity ligand for AMPAR, fluorescein and acyl imidazole. Only when PFQX binds to AMPARs, nucleophilic amino acid residues (Lys, Ser or Tyr) located near ligand-binding domain on AMPAR are attacked acyl imidazole and fluorescein is transferred into AMPARs. After removing excess reagents and resultant ligand moiety, labeled AMPARs can be observed in both live and fixed neuronal cells. The protocol is very simple, no genetic manipulation and additional treatment are required. Because LiveReceptor AMPAR shows no cell membrane permeability, only cell surface AMPARs are labelled. Ref.3 indicates fluorescein-labeled AMPARs by LiveReceptor has little effects on its ion channel capability in neurons.

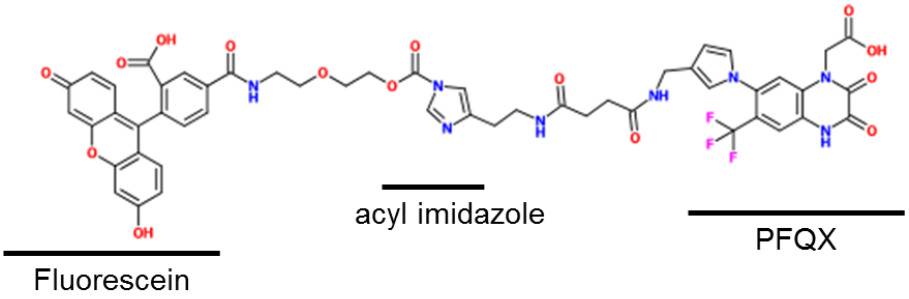


Figure 2. Chemical structure of LiveReceptor AMPAR

Description

Catalog Number: FDV-0018A
Size : 10 µg
Formulation : $C_{51}H_{43}F_3N_8O_{15}$
Molecular weight : 1064.92 g/mol
Visibility : Orange lyophilized powder
Solubility : Soluble in DMSO
*This compound has high 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 AMPAR antagonist

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 3 months at -80ºC. Please make aliquots and avoid freeze and throw. Protected from light.

How to use

General procedure for endogenous AMPAR labeling

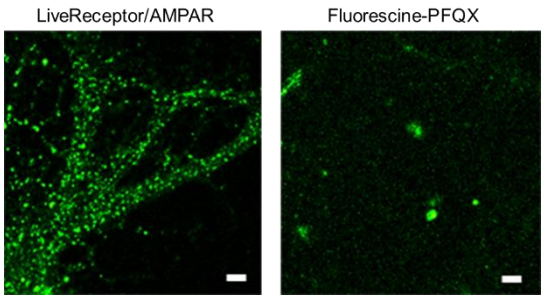
1. Prepare 1 µM of LiveReceptor AMPAR in the appropriate medium
   * Note : Serum-free media are highly recommended. This compound is not stable in the medium. Please prepare assay solution at time of use.
2. Replace media of cultured neurons or slice tissue to LiveReceptor containing medium.
3. Culture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

Detail procedures for various application are described in Ref. 3.

Application data

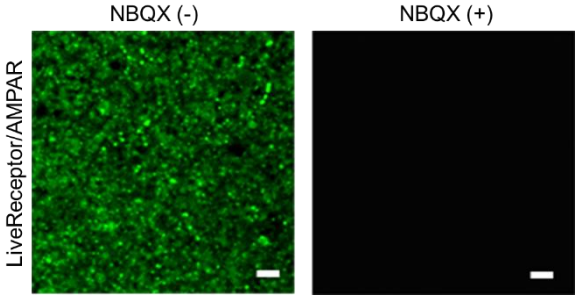
Live cell imaging of labelled endogenous AMPARs in cultured neurons

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 17ºC 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)



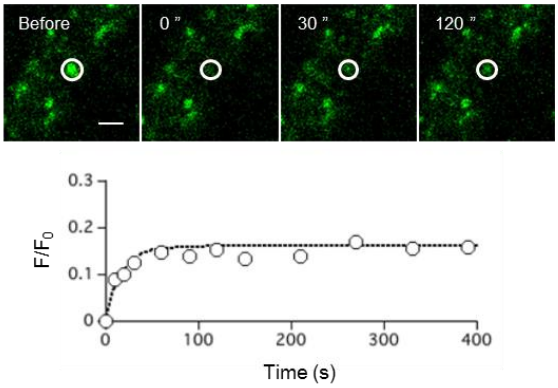
Live cell imaging of labelled endogenous AMPARs in cultured slice tissue

Acutely prepared hippocampal slices were treated with 1 µM of LiveReceptor AMPAR for 1 hour at 17ºC in the absence (in left) or presence (in right) of 10 µM NBQX, a potent inhibitor of AMPAR, and washed out three times with the basal medium. Dendritic spin-like punctual structures were observed on live cells and the signal was clearly disappeared by NBQX. (scal bars, 5 µm)



FRAP analysis for diffusion dynamics of AMPARs

Cultured hippocampal neurons were treated with 1 μM of LiveReceptor AMPAR for 1 hour at 17°C. After washing cells, FRAP (fluorescence recovery after photobleaching) experiment was performed. The recovery ratio and diffusion coefficient were determined to be 16.2% and 0.090 $\mu m^2 s^{-1}$, respectivery. Detail information are described in Ref.3.

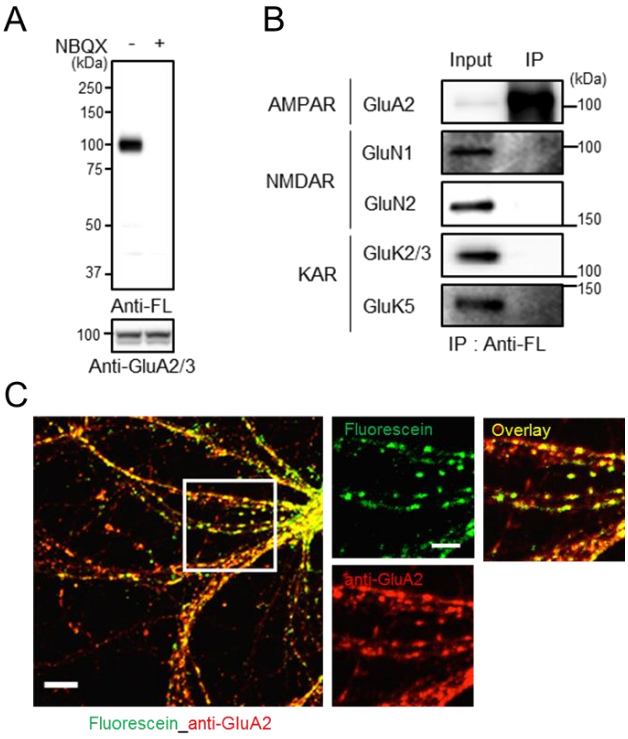


Validation of specificity for AMPARs

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.

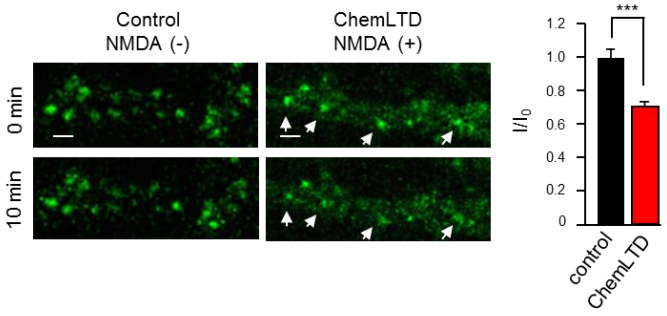
B. Cultured cortical neurons were treated with 1 μM of LiveReceptor AMPAR. After lysis of cultured neurons, the cell lysate was immunoprecipitated with anti-fluorescein antibody. The immunoprecipitates were analyzed by western blot using glutamate receptor-specific antibodies including GluA2 (AMPAR), 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)



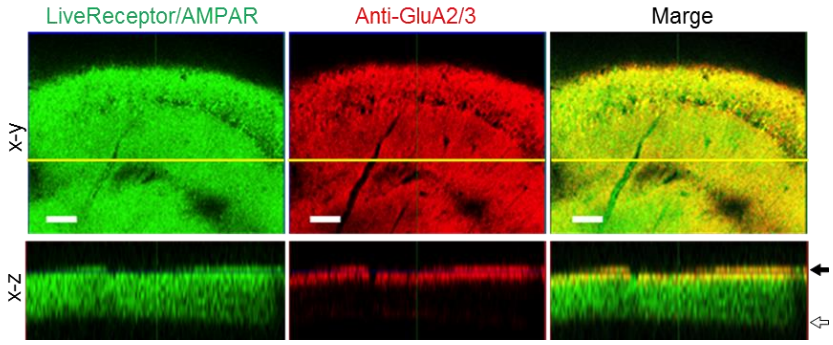
Chemical stimulation-induced synaptic plasticity

Cultured hippocampal neurons labelled with 1 μM of LiveReceptor AMPAR were treated with NMDA for 10 min to chemically induce long-term depression (LTD). Under the LTD condition, AMPAR signals on the spins were significantly reduced by control spines. Based on pH-dependent fluorescein quenching, internalized AMPARs by endocytosis have no fluorescent signal.



Comparison of tissue penetration between LiveReceptor and IgG antibody

Hippocampal slices labelled with 1 μM of LiveReceptor AMPAR were fixed, permeabilized and immunostained using anti-GluA2/3 antibody. x-y image shows fluorescein signal was well overlapped with anti-GluA2/3. Although anti-GluA2/3 antibody stained receptors only in the surface of slice, LiveReceptor AMPAR can penetrated into deep site of slice tissue and labels receptors. (black arrow and white arrow : top and bottom of the slice. Distance of two arrows around 130 μm))



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. Wakayama *et al., Nat. Commun.*, **8**, 14850 (2017). Chemical labeling for visualizing native AMPA receptors in live neurons.

Related products

LiveReceptor $GABA_AR$ <$GABA_AR$ Labeling Reagent>

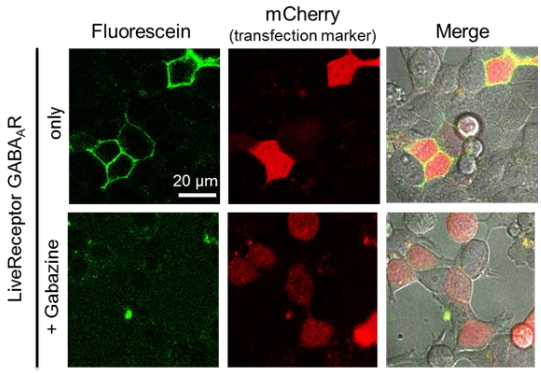
LiveReceptor $GABA_AR$ is a specific labeling reagent for ion channel-type GABA receptor, $GABA_AR$.

Catalog No. FDV-0018B
Size 10 µg

Data example:

Live cell imaging of labelled $GABA_ARs$ 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 and washed out three times with the basal medium. (scale bars, 20 µm)



LiveReceptor mGluR1 <EndogenousmGluR1 Labeling Reagent>

LiveReceptor mGluR1 is a specific labeling reagent for metabotropic glutamate receptor 1, mGluR1. Live imaging of slice cerebellum tissue was validated.

Catalog No. FDV-0018C
Size 10 µg

Data examples

- Live cell imaging of labelled mGluR1 in acute mouse cerebellum brain slice

Acute mouse cerebellum brain slices from 3 week-old mouse treated with 10 nM LiveReceptor mGluR1 in artificial cerebrospinal fluid (ACSF) for 4 hours at RT. After then, slices were washed three times by ACSF and fluorescent signal was observed by epi-fluorescent microscopy. Strong fluorescent signal was observed in molecular layer and Purkinje cells. When the slice was pretreated with FITM, an allosteric mGluR1 inhibitor, fluorescent signal was clearly suppressed.