BioTracker™ Orange-NHS Live Cell pH Dye

Live Cell Acidic pH Dye

Cat. # SCT214

pack size: 1mg

Store at -20ºC

FOR RESEARCH USE ONLY.
NOT FOR USE IN DIAGNOSTIC PROCEDURES.
NOT FOR HUMAN OR ANIMAL CONSUMPTION.

Data Sheet

Background

BioTracker™ Orange-NHS Live Cell pH dye is a chemical probe which reversibly fluoresces upon acidic pH. The probe is modified with N-hydroxysuccimide ester (NHS) which forms a covalent bond with primary amines just by mixing. It can be used for labeling proteins, antibodies and other molecules. For example, it can be applied for visualizing endocytosis of target protein on cell membrane by labeling antibodies that binds to the target protein. This probe binds to primary amines just by mixing. Thus, it can be used to label antibodies and other proteins which have lysine residues. Its fluorescence intensity increases ~20-fold at an acidic pH.
One mg vial of SCT214 can be used to label 10-50 mg antibody protein.

Storage

BioTracker™ Orange-NHS Live Cell pH Dye is shipped in a nitrogen gas-filled vial. Store SCT214 at -20°C, desiccate and protect from light

Note: Centrifuge vial briefly to collect contents at bottom of vial before opening.

Spectral Properties

Absorbance maximum: 544 nm
Emission maximum: 565 nm (Orange)

Quality Control

Purity: ≥ 80% confirmed by LC.

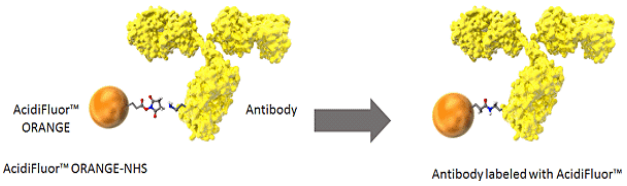

Figure 1: SCT214 binds to primary amines just by mixing. Thus, it can be used to label antibodies and other proteins which have lysine residues, as shown in the figure above.

Product	Target	Reaction	pKa	Ɛ	Φ
SCT214	pH	Reversible	5.3, 6.8	80,000	0.7

Table: Properties of BioTracker™ Orange-NHS Live Cell pH Dye.

Protocol for labeling an antibody

Materials required but not provided

1. 0.1 M sodium bicarbonate ($NaHCO_3$)
2. Anhydrous DMSO
3. Ultrafiltration column, gel filtration or dialysis tube with a suitable molecular size.

Reagent Preparation and fluorescence labeling

1. Dissolve the protein you wish to label in the 0.1 M $NaHCO_3$ buffer to 2-10 mg/mL. Use gel-filtration column to exchange the buffer if the protein is already dissolved in a neutral buffer solution. Refer the manufacture's instruction for the gel-filtration column.
   Note: Amine-containing reagents such as BSA, other proteins, Tris, or glutathione inhibit the labeling reaction. In these cases, purify the protein before the labeling to remove the extra amines.
2. Add 107 µL of DMSO to the 1 mg vial of SCT214, mix well to prepare 10 mM solution. Add this solution to the protein solution prepared in the previous step, at the molar ratio of 2 to 5. Immediately mix the solution by pipetting.
3. Incubate the mixture at 25 ºC for 60 minutes. Protect from light during the incubation. Mix the solution by tapping the tube in every 15 minutes.
4. Remove unreacted dye by using gel-filtration column by exchanging buffer solution with PBS (pH 7.4).

Calculating degree of labeling

The degree of labeling (DOL) by SCT214 can be calculated by the following equation:

- $$DOL = \frac{A_{551}/\epsilon_{551,pH7.4}}{(A_{280} - A_{551} \times CF_{280,551})/\epsilon_{pr}}$$

Where the parameters represent as:
A_{551}, A_{280}: Absorbance of SCT214-conjugated protein at 551 nm and 280 nm, respectively
$CF_{280/551}$: A correction factor for the absorbance (see Table)
$Ɛ_{551, pH7.4}$: An extinction coefficient of SCT214 at 551 nm in pH 7.4 (see Table.)
$Ɛ_{pr}$: An extinction coefficient of a protein at 280 nm. For IgG, 210,000 $M^{-1}cm^{-1}$.

Fluorescence detection

For laser excitation, either 532 nm or 514 nm is appropriate. Fluorescence around 565 nm will be detected. For fluorescent microscopy, general green excitation filter set such as that for Cy3 can be used.

Rev.1.0/2020-04-01/SCT214MD

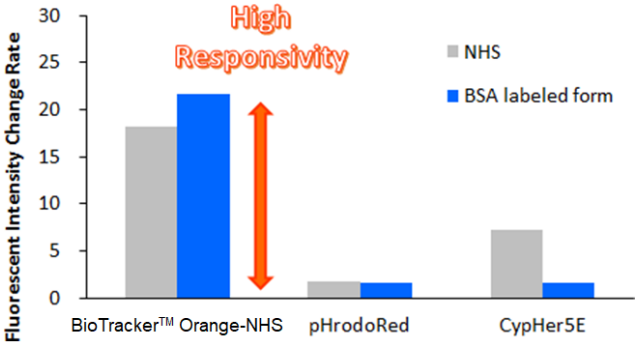

Figure 2: High signal to noise ratio. Fluorescent intensities of three pH probes (BioTracker™ Orange-NHS, pHrodoRed and CypHer5E) and their BSA labeled form were measured in phosphate buffer of pH 5.0 and that of pH 7.4. Intensity of BioTracker™ Orange-NHS (SCT214) shows ~20 times increase at pH 5.0 compared to that at pH 7.4, while other probes showed only 1.8 times and 7.5 times increase in the fluorescence intensities, respectively. Only SCT214 has high pH responsivity and dynamic range as both the NHS and BSA labeled forms.

AcidiFluor™ ORANGE-NHS: λ_{ex} 532 nm / λ_{em} 568 nm
pHrodo™ Red-NHS (Life Technology): λ_{ex} 560nm / /λ_{em} 582 nm
CypHer™ 5E-NHS (GE Healthcare): λ_{ex} 644 nm / /λ_{em} 667 nm

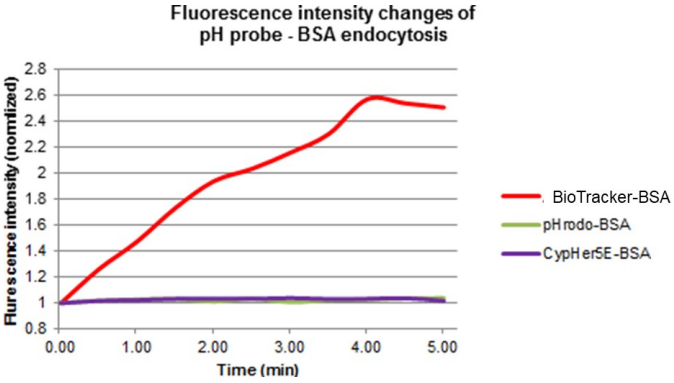

Figure 3: A timecourse of fluorescence intensity changes during endocysosis, measured by choosing a spot on a cell. Fluorescence increase of BioTracker™ Orange-NHS-BSA was most significant compared to BSA labeled with other pH probes.

References

Asanuma D et al. *Acidic-pH-Activable Fluorescence Probes for Visualizing Exocytosis Dynamics*. Angew. Chem Int Ed Engl. 2014 Jun 10; 53(24): 6085-9.

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