

# HaloTag<sup>®</sup> SaraFluor<sup>™</sup> 650B Ligand

Table 1. Product information

Catalog no.	Product name	Amount	Storage upon receipt	Stability
A201-01	HaloTag SaraFluor 650B Ligand	15 nmol × 1	≤-20°C, keep desiccated and protected from light.	1 year (when unopened and stored as described.)
A201-02		15 nmol × 2		

## 1. About HaloTag SaraFluor 650B Ligand

SaraFluor 650B (HMSiR) is a deep-red fluorescence imaging probe for superresolution imaging. It shows spontaneous blinking in a physiological buffer solutions, and is used for single molecule localization microscopy (SMLM). Users can use microscope system for PALM/STORM to use this fluorophore, without additions of oxygen scavengers or irradiation of high-power UV laser. HaloTag SaraFluor 650B Ligand has a chloroalkane that specifically forms covalent bond to HaloTag fusion proteins. This reagent enables easy superresolution imaging of HaloTag fusion proteins in living cells.

※ This product is equivalent to the product which has been provided as “HMSiR-Halo”.

Table 2. Properties of SaraFluor 650B in 0.1M citrate buffer (pH 3.5).

$\lambda_{\text{ex}}$ (nm)	$\lambda_{\text{em}}$ (nm)	$\epsilon$ (M <sup>-1</sup> cm <sup>-1</sup> )
654	669	$1.2 \times 10^5$

### ■ Storage

The reagent is shipped as a dried solid in a nitrogen gas-filled vial. Upon receipt, store the product desiccated and protected from light at ≤-20°C. We provide no warranty for the reagents which was stored as a solution.

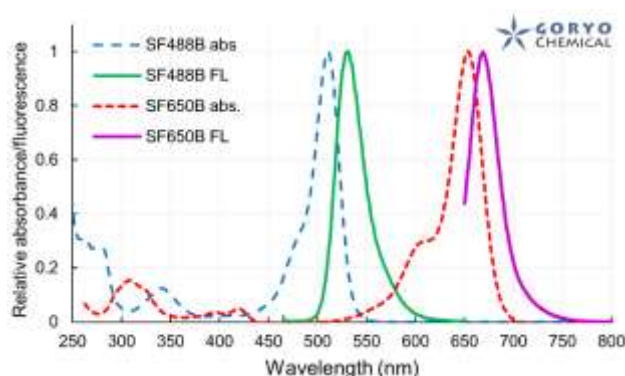
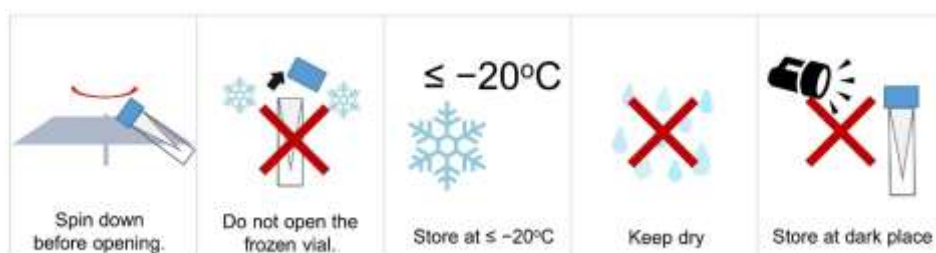


Fig. 1. Spectra of SaraFluor B



## 2. Staining protocol of live cells with HaloTag® SaraFluor 650B Ligand

### ■ Procedure for labeling reaction

1. Dissolve HaloTag® SaraFluor 650B Ligand in DMSO to get 0.1–1 mM stock solution.
  2. On the day before the observation, prepare cells expressing HaloTag® fusion proteins.
  3. Add 0.2–100 nM HaloTag® SaraFluor 650B Ligand to the cultured cells, and incubated overnight at the cultivate condition.
  4. Wash cells with the culture medium, and replace the cells on the glass bottom dish.
  5. Observe the cells with STORM about 3 hours after the replacement.
- ※ Appropriate concentration and incubation time varies depending on the HaloTag® protein species and amounts.

### ■ Fluorescent observation

The intensity of 647 nm excitation laser for the evanescent field is 100 W/cm<sup>2</sup> when using N-STORM (Nikon). 692/40 nm bandpass emission filter (Semrock) is usable for the observation. Irradiation of 405 nm laser is not necessary. Observe cell in PBS without any additives. Capture hundreds-to-10-thousands of images to construct a superresolution image by following the instructions of the microscope.

### ■ References

S.N. Uno, M. Kamiya, T. Yoshihara, K. Sugawara, K. Okabe, M.C. Tarhan, H. Fujita, T. Funatsu, Y. Okada, S. Tobita, Y. Urano (2014) *Nat. Chem.* **6**: 681-689. [DOI:10.1038/nchem.2002](https://doi.org/10.1038/nchem.2002)

F.C. Chien, C.Y. Linb, G. Abrigoa (2018) *Phys. Chem. Chem. Phys.* **20**: 27245-27255.  
[DOI:10.1039/C8CP02942C](https://doi.org/10.1039/C8CP02942C)

Table 3. Related Products

Catalog no.	Product name	Major applications
A202-01	SaraFluor 650B goat anti-mouse IgG	Superresolution microscopy by immunocytochemistry.
A203-01	SaraFluor 650B goat anti-rat IgG	Superresolution microscopy by immunocytochemistry.
A204-01	SaraFluor 650B goat anti-rabbit IgG	Superresolution microscopy by immunocytochemistry.
A208.01	SaraFluor 650B-NHS	Superresolution imaging probes which can label primary amines. In proteins, it can label lysine residues.
A209-01	SaraFluor 650B-MLI	Superresolution imaging probes which can label thiols. In proteins, it can label cysteine residues.
A308-01	HaloTag® SaraFluor 650T Ligand	For STED imaging of HaloTag®-labeled proteins.
ST1008-11	SaraFluor 650-NHS	For STED imaging. This probe can label primary amines.

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