

For research use only

SaraFluor™ 650B goat IgG series

Table 1. Product information

| Catalog no. | Product name | Amount | Storage upon receipt | Stability |
|-------------|-------------------------------------|--------|---|---|
| A202-01 | SaraFluor 650B goat anti-mouse IgG | 100 µg | Store at -15°C to -20°C, protecting from light. | 1 year (when unopened and stored as described.) |
| A203-01 | SaraFluor 650B goat anti-rat IgG | | | |
| A204-01 | SaraFluor 650B goat anti-rabbit IgG | | | |

1. About SaraFluor 650B labeled antibodies

SaraFluor 650B labeled goat IgG series includes secondary antibodies for superresolution imaging using PALM/STORM microscopy. SaraFluor 650B is a fluorescent probe which shows spontaneous blinking in physiological buffer conditions. It can be observed without adding thiols, oxygen scavengers as well as without irradiating high-power laser.

※These products are equal with HMSiR-labeled antibodies those we have sold until 2018.

Table 2. Specifications of SaraFluor 650B goat antibodies

| λ_{ex} (nm) | λ_{em} (nm) | ϵ ($M^{-1}cm^{-1}$) |
|---------------------|---------------------|--------------------------------|
| 654 | 669 | 1.2×10^5 |

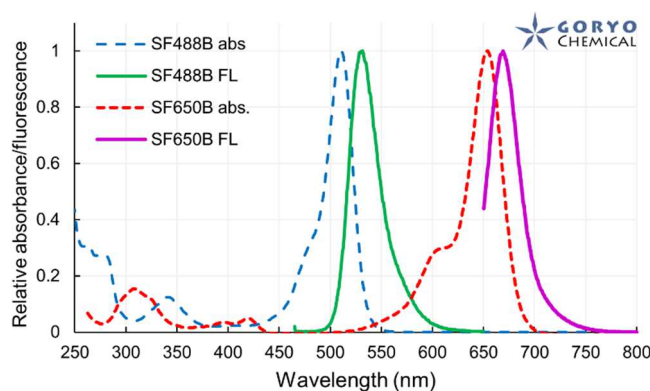
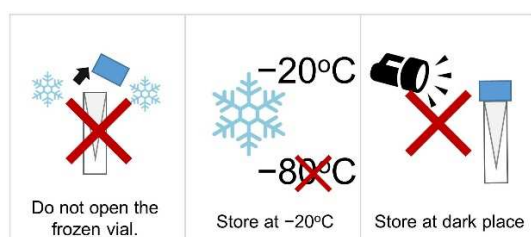


Fig. 1. Excitation/emission spectra of SaraFluor B.

■ Storage

The product is shipped either in blue ice or in dry ice. Upon receipt, store the product at -20°C. Avoid storing below -25 °C, at which the product containing 50% glycerol freezes. Alternatively, you can store the frozen product which has been shipped in dry ice, at -80°C until the first use. Keep -20°C after the product was once defrosted. Avoid freeze-thaw cycle.

Addition of final 0.05% sodium azide may protect the product from bacterial contamination.

2. An example of cell staining and observation

■ Materials required but not provided

- Washing buffer (1×PBS pH 7.4, or other appropriate buffer).

■ Example cell imaging procedure

1. Fix cells with appropriate fixation solution (ex. 3% paraformaldehyde, 0.2% glutaraldehyde in PBS) for 5 minutes at 37°C.
2. Remove the solution and add –20 °C methanol for postfixation. Incubate for 5 minutes at –20 °C.
3. Replace the solution with PBS supplemented with 1% Triton X-100. Incubate for 5 minutes at room temperature (RT).
4. Replace the solution with PBS supplemented with 100 mM glycine to quench the aldehydes.
5. Replace the solution with PBS containing 1% BSA for blocking. Incubate for 15 minutes at RT.
6. Replace the solution with PBS containing primary antibody. Incubate for 1 hour at RT.
7. Wash the cells 3 times with PBS, and add 10–30 µg/mL of this SaraFluor 650B labeled antibody (as a secondary antibody) to incubate for 1 hour at RT.
8. Remove the antibody solution, wash twice with PBS and observe the cells with an appropriate microscope (for PALM or STORM). Optionally, replacement to mounting media with antifade reagent can reduce the photobleach.
9. 647 nm laser is appropriate for excitation. Typically, about 30% of laser power used for the observation of Alexa Fluor® 647 is suitable for SaraFluor 650B observation (ex. 100 W/cm²). No need to irradiate 405 nm laser, nor addition of reducing reagents. Capture 1000–30,000 images to obtain single superresolution image.

* Optimizations of fixation solution, and the concentrations of antibodies depending on the cell types and antigens are required.

Table 2. Related Products

| Catalog no. | Product name | Major applications |
|-------------|--------------------------------|---|
| A201-01 | HaloTag® SaraFluor 650B ligand | For PALM/STORM superresolution imaging of HaloTag® fused proteins. Red laser excitation. |
| A208-01 | SaraFluor 650B-NHS | SaraFluor 650B that can be conjugated with primary amine residues, such as that of lysine residues in proteins. Red laser excitation. |
| A209-01 | SaraFluor 650B-maleimide | SaraFluor 650B that can be conjugated with thiols, such as that of cysteine residues in proteins. Red laser excitation. |
| A218-01 | SaraFluor 488B-NHS | SaraFluor 488B that can be conjugated with primary amine residues, such as that of lysine residues in proteins. Blue laser excitation/green fluorescence. |
| A308-01 | HaloTag® SaraFluor 650T Ligand | For STED superresolution imaging of HaloTag® fused proteins. Red laser excitation. |

HaloTag® is a trademark of Promega Co.