# LipiBright™ 530 SMCy3.5: Fluorogenic Lipid Droplet Probe

V. 2.4

**Cat. # MG11-70 (70 nmoles)**

Lot: 015

Upon arrival store at 4°C (desiccated)

See below for storage after reconstitution

## Background

Intracellular lipid-rich organelles known as lipid droplets have been shown to be important both physiologically and pathologically. The LipiBright™ probes consists of bright, fluorogenic live cell dyes that stain lipid droplets. LipiBright™ probes fluoresce 300 to 1000 times brighter in an oily/lipid environment compared to aqueous solutions[1]. The probes are available in three different colors; yellow: LipiBright™ 530 SMCy3.5 (Em = 570-610 nm), far red: LipiBright™ 650 SMCy5.5 (Em = 680-720 nm) and near-infrared: LipiBright™ 740 SMCy7 (Em = 770-810 nm). LipiBright probes are photostable and possess narrow absorption and emission bands, a distinct advantage over the traditional Nile Red probe; thus, multicolor imaging is possible. These probes exhibit ideal characteristics for microscopy and have been validated with multiple techniques including epifluorescent (widefield), confocal, and two photon[1]. With high GM values (2400 to 13300), the LipiBright SMCy3.5 and SMCy5.5 probes have been confirmed to work with two photon microscopy to work in multiple models including cells and tissues[1].

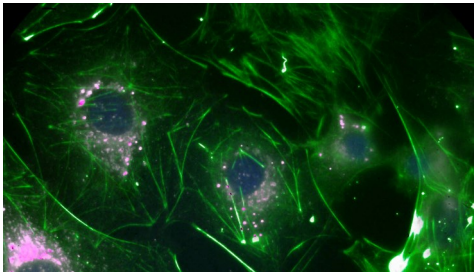
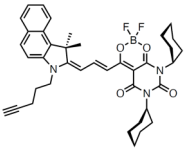
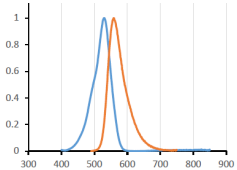


**Figure 2.** Swiss3T3 cell monolayer labeled with LipiBright™ SMCy3.5. Wide-field fluorescent imaging of live Swiss3T3 labeled with LipiBright™ SMCy3.5 (Pink), a live cell actin cytoskeleton stain (Green, SiR-Actin), and a live cell DNA probe (Blue, SPY505-DNA). Cells were imaged as described in **Application 1** methods. LipiBright™ signals were visualized with a TRITC filter set, a digital CCD camera, and 40x objective.

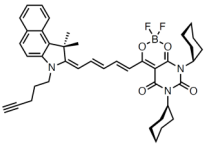
SMCy3.5 mol structure

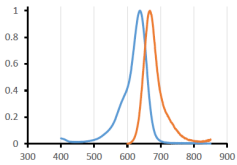


Emission and Excitation spectra

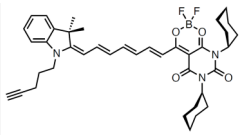


SMCy5.5 mol structure





SMCy7.0 mol structure



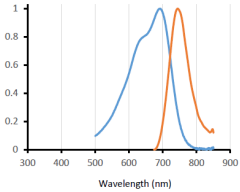



**Figure 1. Structure and range of fluorescence.** A - Structure of LipiBright SMCy3.5, 5.5 and 7.0, note the increasing length of the polymethyne chain. B - Normalized absorption spectra (blue) and normalized emission spectra (orange) of SMCy3.5, SMCy5.5 and SMCy7.5 in Labrafac oil (Gattefossé, Saint Priest, France). LipiBright™ is a trademark of CNRS, France.

## Material

LipiBright™ SMCy3.5 can be visualized using a TRITC or similar filter set. As measured in DMSO, the absorption max of LipiBright™ SMCy3.5 in oil is 530 nm, with an emission maximum of 558 nm, an extinction coefficient of $110x10^3$, and LipiBright™ SMCy3.5 is supplied as a lyophilized pellet. Avoid contact with LipiBright™ by wearing appropriate PPE and dispose of according to local regulations and policies.

## Storage and Reconstitution

The lyophilized product is stable at 4°C (<10% humidity) for 6 months and should be protected from light. To reconstitute, briefly centrifuge to collect the product at the bottom of the tube.

LipiBright™ should be reconstituted with 200 µl of anhydrous DMSO to create a 340 µM stock solution for cell imaging or with 40 µl of anhydrous DMSO to create a 1.5 mM stock suitable for tissue or small organism imaging. After reconstitution the solution should be stored at -20°C where it is stable for 6 months. Once reconstituted, allow product to warm to room temperature before opening tube.

## Important Technical Notes

A. Dilute solutions of LipiBright™ in aqueous media must be used immediately (<20 sec), as LipiBright™ will precipitate and/or bind to tube walls.

B. Serum can reduce LipiBright™ staining efficiency. When possible LipiBright™ staining should take place in the absence of serum. Optimally, the imaging cell media is serum-free media, reduced serum media, or PBS. In lieu of serum removal, the concentration of LipiBright™ should be increased.

C. Samples incubating in LipiBright™ solution should be protected from light.
D. LipiBright™ is non-toxic and live cells can be returned to normal cell media following labeling, and they will remain-labeled for 2-4 days.
E. For tissues and small organisms an initial labeling concentration of 1 µM is recommended. For cell culture an initial labeling concentration of 50 nM is recommended depending on application.
F. After uptake, LipiBright probes will be transported to lipid globules via the endosome pathway and Golgi apparatus, this process may take a few hours, 4-6h, and hence the most defined staining is observed after this time.
G. For fixed cell applications: When co-labeling with antibodies that require permeabilization do not use Triton-X, but replace it with saponin or digitonin which are milder (see Ohsake et al. 2005).
H. LipiBright™ is fully compatible with 4% paraformaldehyde (PFA) or 1% glutaraldehyde. Staining should be performed before fixation i.e. in live cells, in order to allow its localization to lipid droplets which takes a 4-6h.

**Application 1:** Labeling the lipid droplets of live cells in culture.

**Reagents**
1. LipiBright™ SMCy3.5 (Cat. # MG11).
2. Semi-confluent Swiss 3T3 cells grown on 25mm X 25mm sq glass coverslips.
3. Imaging medias: Serum-free DMEM media.

**Equipment**
1. Fluorescent microscope with a TRITC or Cy3.5 filter block, e.g. excitation filter at 535 +/-20 nm and emission filter at 585 +/-20 nm.
2. Digital camera.

**Method**
1. Cells should be seeded onto imaging-appropriate glass or plastics and grown according to cell line requirements to semi-confluency.
2. Aspirate media from cells and wash two times with serum free media. Do not allow the cells to dry.
3. Prepare the probe solution by diluting 4 µl of 340 µM LipiBright™ stock in to 4 ml serum free media to create a 340 nM working solution and mix thoroughly. Work quickly (<20 secs) as the probes will begin to aggregate reducing labeling efficiency.
4. Add diluted probe solution to cells and swirl gently until covered.
5. Incubate for 4-6 hours at 37°C, 5% $CO_2$ and 95-100% humidity.
6. Wash coverslips gently twice with serum free media without probe. Do not pipette wash solution directly onto cells.
7. Replace with serum containing media without probe.
8. Proceed with imaging.

**Product Citations/Related Products**
1. Collot M. et al. 2018. Ultrabright and Fluorogenic Probes for Multicolor Imaging and Tracking of Lipid Droplets in Cells and Tissues. JACS, 140, p.5401-5411.
2. Ohsaki Y. et al. 2005. Fixation and permeabilization protocol is critical for the immunolabeling of lipid droplet proteins. Histochem Cell Biol,124(5), p.445-52. doi: 10.1007/s00418-005-0061-5.

For the latest protocols, citations and related products please visit www.cytoskeleton.com/memglow.

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