Product Information

LipidSpot™ Lipid Droplet Stains

Product	Catalog no.	Size
LipidSpot™ 488 Lipid Droplet Stain, 1000X in DMSO	70065-T	20 uL
	70065	125 uL
LipidSpot™ 610 Lipid Droplet Stain, 1000X in DMSO	70069-T	20 uL
	70069	125 uL

Storage and Handling

Store at 4°C, protected from light. Product is stable for at least 12 months from date of receipt when stored as recommended.

Spectral Properties

Dye	Ex/Em	Detection channel
LipidSpot™ 488	427/585 nm	GFP, FITC
LipidSpot™ 610	610/663 (in vegetable oil) ~592/638 nm (in cells)	Texas Red® or Cy®5

See Figures 1-2 for spectra. The spectral properties of LipidSpot™ dyes are highly sensitive to their environment and vary in different solvents compared to cells.

Product Description

Intracellular lipid droplets are cytoplasmic organelles involved in the storage and regulation of triglycerides and cholesterol esters. LipidSpot™ dyes rapidly stain lipid droplets in live or fixed cells with no wash step and minimal background staining of cellular membranes or other organelles. Cells also can be fixed and permeabilized after staining.

LipidSpot™ 488 has excitation around 430 nm, and can be excited equally well at 405 nm or 488 nm. In cells, it stains lipid droplets with bright green fluorescence.

LipidSpot™ 610 has excitation/emission at ~592/638 nm in cells. Lipid droplet staining in cells is optimally detected in the Texas Red® channel, but is also bright in the Cy®3 and far-red Cy®5 channels. Therefore, we don't recommend pairing LipidSpot™ 610 with other red or far-red probes.

Protocols

Live cell staining

1. Dilute LipidSpot™ Lipid Droplet Stain to 1X in complete cell culture medium or other buffer if desired. The dye concentration may be optimized if needed.
2. Incubate cells with the stain at 37°C for 30 minutes or longer, protected from light. No obvious cytotoxicity of the dye has been observed with incubation times up to 24 hours.
3. Image fluorescence in the appropriate detection channel (see Spectral Properties). Washing before imaging is optional.
4. Cells can be fixed in formaldehyde after staining. Staining also can withstand permeabilization by 0.1% Triton X-100, although permeabilization may alter lipid droplet morphology.

Staining of fixed cells

1. Fix cells with a formaldehyde-based fixative. Alcohol fixation is not recommended. Cells can be permeabilized with 0.1% Triton X-100 before staining, although permeabilization may alter lipid droplet morphology.
2. Dilute LipidSpot™ Lipid Droplet Stain to 1X in PBS or other buffer. The dye concentration may be optimized if needed.
3. Incubate cells with stain at room temperature for 10 minutes or longer, protected from light.
4. Image fluorescence in the appropriate detection channel (see Spectral Properties). Washing before imaging is optional.

   Note: we do not recommend using antifade mounting medium with LipidSpot™ dyes, because it can reduce staining and increase background.

Optional: Lipid droplet induction in cultured cells

Oleic acid complexed to BSA can be used to induce lipid droplet formation in cultured cells as a positive control for LipidSpot™ staining.

Materials required but not provided:

Oleic acid
50% ethanol in dH_2O
Bovine serum albumin, fatty acid free (defatted BSA)

Cell treatment protocol:

1. Warm oleic acid to 37°C until it is completely liquefied.
2. Dilute oleic acid to 150 mM in 50% ethanol by mixing 47 uL oleic acid with 953 uL 50% ethanol. Vortex to mix. Oleic acid will form a cloudy white suspension. Diluted oleic acid can be stored at 4°C. Before use, warm to 37°C and vortex to resuspend.
3. Dissolve defatted BSA at 100 mg/mL in dH_2O. BSA solution can be stored at -20°C.
4. Prepare oleic acid/ BSA complex on the day the cells are to be treated. Combine equal volumes of 150 mM oleic acid and 100 mg/mL defatted BSA in dH_2O and mix well by pipetting up and down. Incubate the mixture at 37°C for 1 hour. The mixture will be cloudy white and viscous.
5. Dilute the oleic acid/BSA complex 1:150 in complete cell culture medium for a final concentration of 0.5 uM oleic acid.

   Note: The cytotoxicity of oleic acid may vary between cell types, so the concentration of oleic acid/BSA complex may require optimization.
6. Incubate cells with oleic acid overnight at 37°C. Include untreated cells as a negative control. After oleic acid treatment, vesicle-like droplets should be visible in the cytoplasm by phase contrast microscopy.
7. Proceed with LipidSpot™ staining of cells. Cells also can be fixed with formaldehyde and stored at 4°C in PBS prior to staining.

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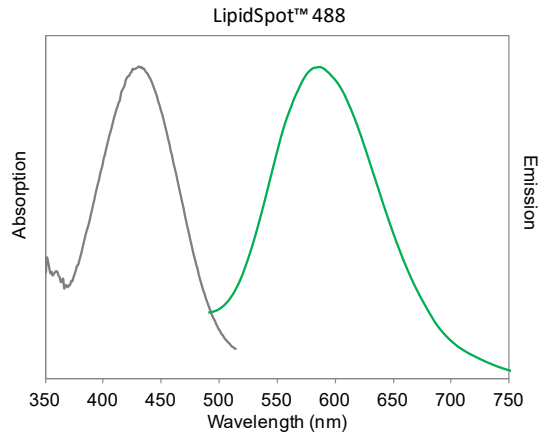

Figure 1. Absorption and emission spectra of LipidSpot™ 488 dye in vegetable oil. The spectral properties of the dye in cells are similar.

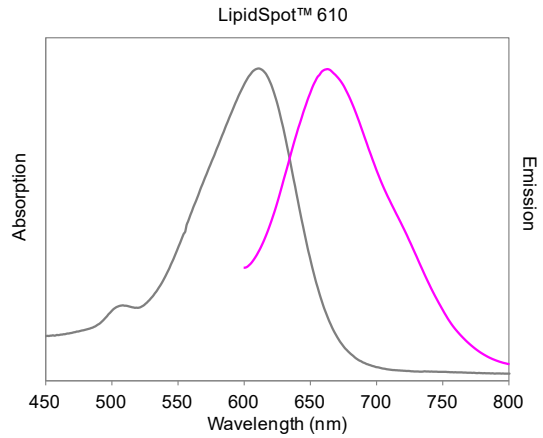

Figure 2. Absorption and emission spectra of LipidSpot™ 610 dye in vegetable oil. In cells, the excitation and emission spectra are green-shifted (left-shifted) 20-25 nm.

Related Products

Catalog number	Product
30088	CellBrite™ Fix 555 Membrane Stain
30089	CellBrite™ Fix 640 Membrane Stain
30024	CellBrite™ Blue Cytoplasmic Membrane Labeling Kit
30021	CellBrite™ Green Cytoplasmic Membrane Dye
30022	CellBrite™ Orange Cytoplasmic Membrane Dye
30023	CellBrite™ Red Cytoplasmic Membrane Dye
30070	CellBrite™ NIR 680
30077	CellBrite™ NIR 750
30078	CellBrite™ NIR 770
30079	CellBrite™ NIR 790
40083	NucSpot® 470 Nuclear Stain for dead or fixed cells
40081	NucSpot® Live 488 Nuclear Stain
40082	NucSpot® Live 650 Nuclear Stain
40060	RedDot™1 Far-Red Nuclear Stain
40061	RedDot™2 Far-Red Nuclear Stain
70066	LysoView™ 405
70067	LysoView™ 488
70061	LysoView™ 540 Lysosome Stain
70058	LysoView™ 633 Lysosome Stain
70059	LysoView™ 650 Lysosome Stain
70070	MitoView™ 405 Mitochondrial Dye
70054	MitoView™ Green Mitochondrial Dye
70055	MitoView™ 633 Mitochondrial Dye
70064	ViaFluor® 405 Live Cell Microtubule Stain
70062	ViaFluor® 488 Live Cell Microtubule Stain
70063	ViaFluor® 647 Live Cell Microtubule Stain

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