Product Information

LipidSpot™ Lipid Droplet Stains

Product	Cat. 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 (nm)	Detection Channel
LipidSpot™ 488	431/587	GFP, FITC
LipidSpot™ 610	611/663 (in vegetable oil) ~592/638 (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 peak excitation around 430 nm and can be excited equally well at 405 nm or 488 nm (see Figure 1 on Page 2) and detected using GFP or FITC channels. In cells, it stains lipid droplets with bright green fluorescence.

LipidSpot™ 610 has peak excitation/emission at ~592/638 nm in cells (see Figure 2 on Page 2). 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.

Experimental Protocols

1. (Optional) Cell treatment protocol

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 (for cell treatment protocol only)

- Oleic acid (≥99%)
- 50% ethanol in dH_2O
- Bovine serum albumin, fatty acid free (defatted BSA)

1.1 Warm oleic acid to 37°C until it is completely liquefied.

1.2 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.

1.3 Dissolve defatted BSA at 100 mg/mL in dH_2O. BSA solution can be stored at -20°C.

1.4 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.

Note: Gelling of oleic acid/ BSA mixture may occur during incubation. Suspend the mixture as best as possible by pipetting up and down and avoid introducing any large aggregates to the cells in step 5. There is normally enough lipid in suspension to properly induce droplet formation in cells.

1.5 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.

1.6 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.

Note: After treatment, proceed to the live cell staining protocol. If you plan to fix cells prior to staining, proceed to the fixed cell staining protocol.

2. Live cell staining

2.1 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.

2.2 Replace cell culture medium with staining solution and incubate 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.

2.3 Image fluorescence in the appropriate detection channel (see Spectral Properties). Washing before imaging is optional.

2.4 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.

Note: We do not suggest using a mounting medium with glycerol or organic solvents to mount cells stained with LipidSpot™ Dyes. This can reduce or alter staining and increase background. If using a mounting medium is required, we recommend imaging as soon as possible after mounting (no more than 24h later) to avoid changes in the staining pattern. We recommend imaging directly in PBS (or other aqueous buffers). Antifade may be added to the buffer. Coverslips should be mounted using PBS and sealed with a suitable coverslip sealant such as CoverGrip™ or nail polish. Stained samples can be stored in PBS at 4°C for several weeks or longer.

3. Staining of fixed cells and tissue

3.1 Fix cells with a formaldehyde-based fixative. Alcohol fixation is not recommended as lipid droplet morphology is not well retained with alcohol fixation. Cells can be permeabilized with 0.1% Triton® X-100 before staining, although permeabilization may alter lipid droplet morphology.

3.2 Dilute LipidSpot™ Lipid Droplet Stain to 1X in PBS or other buffer. The dye concentration for optimal staining will need to be determined empirically.

3.3 Incubate cells in staining solution at room temperature for 10 minutes or longer, protected from light.

3.4 Image fluorescence in the appropriate detection channel (see Spectral Properties). Washing before imaging is optional. See the note above regarding proper mounting media.

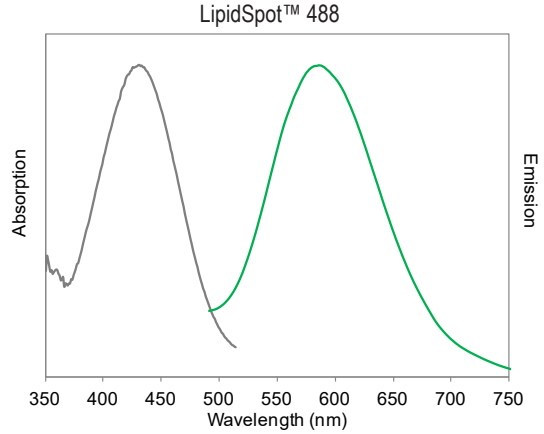

Figure 1. Absorption and emission spectra of LipidSpot™ 488 Dye in vegetable oil. The spectral properties of the dye in cells are similar.

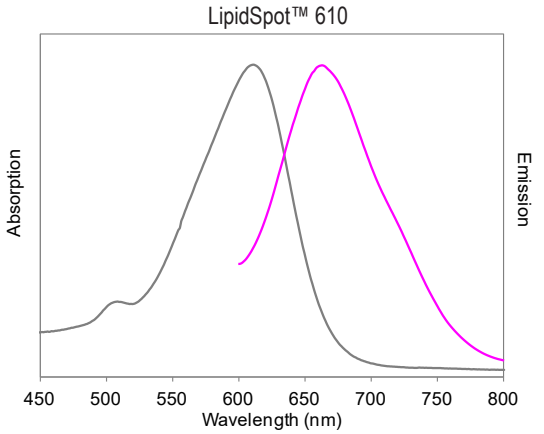

Figure 2. Absorption and emission spectra of LipidSpot™ 610 Dye in vegetable oil. In cells, the excitation and emission spectra are blue-shifted (left-shifted) 20-25 nm.

Frequently Asked Questions

Questions	Solutions
Can LipidSpot™ be used for staining tissue?	We don't generally recommend LipidSpot™ for tissue staining due to the poor preservation of droplets. However, customers have reported that this can work. It seems that imaging immediately after preparation and imaging without a mounting medium increase the likelihood of success.
Can LipidSpot™ be used to stain cells for flow cyometry analysis?	While the dyes have spectral profiles that should be compatible with standard flow cytometry instrument configurations, we don't have any data validating their use for this application.

Related Products

Cat. No.	Product
30021-30024	CellBrite® Cytoplasmic Membrane Dyes
30070...30079	CellBrite® NIR Cytoplasmic Membrane Dyes
29021...29077	Wheat Germ Agglutinin (WGA) Conjugates
30088-30090	CellBrite® Fix Membrane Stains
30092...30104	MemBrite® Fix Cell Surface Staining Kits
30105-30109	CellBrite® Steady Membrane Staining Kits
40083...41038	NucSpot® Nuclear Stains for Dead or Fixed Cells
40081-40082	NucSpot® Live Nuclear Stains
40082	NucSpot® Live 650 Nuclear Stain
40060	RedDot™1 Far-Red Nuclear Stain
40061	RedDot™2 Far-Red Nuclear Stain
70058...70086	LysoView™ Lysosome Stains
70054...70075	MitoView™ Mitochondrial Dyes
70062-70064	ViaFluor® Live Cell Microtubule Stains

Please visit our website at www.biotium.com for information on our life science research products, including environmentally friendly EvaGreen® qPCR master mixes, fluorescent CF® Dye antibody conjugates and reactive dyes, apoptosis reagents, fluorescent probes, and kits for cell biology research.