BODIPY(493/503) Staining for Lipid Droplets

Fluorescent staining protocol for visualizing cellular lipid droplets

Overview

BODIPY 493/503 is a lipophilic fluorescent dye used to visualize neutral lipid storage organelles (lipid droplets) in cells. This protocol provides methods for staining both adherent and suspension cells.

Applications:

- Lipid metabolism studies
- Adipocyte differentiation
- Lipid droplet dynamics
- Oleic acid or fatty acid treatment experiments
- Metabolic disease research

Fluorescence Properties

- **Excitation:** 493 nm
- **Emission:** 503 nm
- **Color:** Green fluorescence
- **Compatible with:** FITC/GFP filter sets

Key Features

- Rapid staining (1-2 hours)
- Live or fixed cells
- Compatible with DAPI nuclear staining
- Minimal background fluorescence

Safety Considerations

- BODIPY is dissolved in DMSO - handle with care

- DMSO can facilitate absorption of other chemicals through skin
- Wear gloves and work in biosafety cabinet when handling cells
- Dispose of fluorescent dye waste properly

Required Materials

Reagents

- BODIPY 493/503 (Thermo Fisher, Cat# D3922)
- DMSO (dimethyl sulfoxide), sterile
- HBSS (Hank's Balanced Salt Solution), sterile
- Fluoromount mounting medium with DAPI
- Oleic acid (optional, for lipid induction)
- Culture medium appropriate for your cells

Equipment

- Biological safety cabinet (BSC)
- 37°C CO_2 incubator
- Fluorescence microscope (FITC/GFP filter)
- Centrifuge (for suspension cells)
- Cytocentrifuge (for suspension cells)
- Microscope slides (positively charged)
- Coverslips
- Clear nail polish (for sealing)

Stock Solutions

BODIPY Stock Solution (10 mM):

- Dissolve BODIPY in DMSO to 10 mM
- Store at -20°C protected from light
- Stable for several months

Working Solution (1 µM):

- Dilute stock 1:10,000 in sterile HBSS
- Prepare fresh on day of use

Optional Materials

- Oleic acid-BSA conjugate (for lipid loading)
- Chamber slides or coverslip dishes
- Paraformaldehyde 4% (for fixation)
- PBS (phosphate buffered saline)

Select Cell Type:

Adherent Cells | Suspension Cells

BODIPY Staining - Adherent Cells

> Note: Cells can be grown directly on coverslips, chamber slides, or in culture dishes.

1. Cell Preparation (Optional Lipid Loading)

Mark Done

- Grow cells to desired confluence in culture vessel
- **Optional:** Treat with oleic acid (typically 100-400 µM) for 12-24 hours to induce lipid droplet formation
- Cells can be used at any confluence, typically 50-80%

2. Wash Cells

Mark Done

- Aspirate culture medium
- Wash cells twice with sterile, room temperature HBSS
- Remove all traces of serum-containing medium (can increase background)

3. Prepare BODIPY Staining Solution

Mark Done

- Dilute BODIPY stock solution (10 mM in DMSO) in sterile HBSS
- **Final concentration: 1 µM** (1:10,000 dilution)
- Example: For 10 mL, add 1 µL BODIPY stock to 10 mL HBSS
- Mix thoroughly and protect from light

4. Stain Cells

Mark Done

- Add 1 µM BODIPY staining solution to cells (enough to cover cells)
- Incubate for **1-2 hours at 37°C**

- Protect from light during incubation
- Use foil or dark incubator chamber

5. Post-Staining Wash

Mark Done

- Remove staining solution
- Wash cells twice with HBSS to remove unbound dye
- Final wash should be thorough to minimize background

6. Mounting with DAPI

Mark Done

- Add 1-2 drops of Fluoromount + DAPI to cells
- If using coverslips, carefully place on microscope slide
- If imaging dish directly, proceed to microscopy
- Allow mounting medium to cure overnight at 4°C in dark
- Optional: Seal coverslip edges with clear nail polish

BODIPY Staining - Suspension Cells

> This protocol includes cytospinning to adhere cells to slides for imaging.

1. Harvest Cell Suspension

Mark Done

- Collect cell suspension in sterile centrifuge tube
- Optional: Pre-treat with oleic acid if desired

2. Pellet Cells

Mark Done

- Centrifuge at **1000 RPM for 5 minutes**
- Carefully remove supernatant without disturbing pellet

3. Wash Cells

Mark Done

- Resuspend pellet in 2-3 mL room temperature HBSS
- Centrifuge at 1000 RPM for 5 minutes
- Remove supernatant
- **Repeat wash 2x total** (2 washes)

4. Stain Cells

Mark Done

- Resuspend cell pellet in 1 µM BODIPY solution in HBSS
- Incubate at **37°C for 1-2 hours**
- Protect from light during incubation (cover with foil)

5. Post-Staining Wash

Mark Done

- Centrifuge at 1000 RPM for 5 minutes
- Remove supernatant
- Wash twice with HBSS (centrifuge between washes)

6. Prepare for Cytospin

Mark Done

- After final wash, resuspend pellet in small volume of HBSS
- Adjust concentration so final cell suspension **does not exceed 10^5 cells/mL**
- Mix thoroughly to ensure single-cell suspension

7. Cytospin

Mark Done

- Use positively charged microscope slides
- Load **maximum 500 µL** of cell suspension into cytospin chamber
- Centrifuge at **500 × g for 5 minutes**
- Remove slides and let dry briefly (2-3 minutes)
- Check for cell spots under microscope

8. Mount and Seal

Mark Done

- Add a drop of Fluoromount + DAPI mounting medium to cell spot
- Carefully place coverslip over mounting medium
- Let slides cure overnight at 4°C protected from light
- Seal coverslip edges with clear nail polish to prevent drying
- Allow nail polish to dry completely before imaging

Fluorescence Imaging

BODIPY Channel (Lipid Droplets)

- **Filter:** FITC/GFP
- **Excitation:** 488-495 nm
- **Emission:** 500-520 nm
- **Appearance:** Bright green puncta

DAPI Channel (Nuclei)

- **Filter:** DAPI/Hoechst
- **Excitation:** 340-365 nm
- **Emission:** 420-480 nm

• **Appearance:** Blue nuclei

Imaging Tips

- Use oil immersion objective (40× or 63×) for high-resolution imaging of lipid droplets
- Lipid droplets appear as distinct bright green punctate structures in cytoplasm
- Set exposure time to avoid saturation of brightest droplets
- Image DAPI first (to locate cells) then BODIPY channel
- Use Z-stacking for 3D reconstruction of lipid droplet distribution
- Minimize photobleaching by using minimal light intensity
- Store slides at 4°C protected from light between imaging sessions

Expected Results:

• Basal cells: Few small lipid droplets
• Oleic acid-treated cells: Numerous large lipid droplets throughout cytoplasm
• Adipocytes: Very abundant large lipid droplets
• Size range: 0.5-5 µm diameter (typically 1-2 µm)

Troubleshooting

Problem	Possible Cause	Solution
No or weak signal	Low lipid content BODIPY degraded Incorrect filter	Treat cells with oleic acid to induce lipid droplets Use fresh BODIPY stock Verify using FITC/GFP filter set
High background	Insufficient washing Too much dye	Increase number of washes Reduce BODIPY concentration to 0.5 µM
Uneven staining	Poor mixing Inadequate incubation	Mix staining solution thoroughly Extend incubation to 2 hours Ensure even distribution of solution

Problem	Possible Cause	Solution
Cells detached during staining	Harsh washing Prolonged incubation	Wash more gently Reduce incubation time Use chamber slides for better adhesion
Photobleaching	Excessive light exposure	Reduce light intensity Use anti-fade mounting medium Minimize time under microscope
Poor cytospin results	Cell concentration too high/low Clumped cells	Adjust to 10^4-10^5 cells/mL Pipette thoroughly to create single-cell suspension Filter through 40 µm mesh

Additional Notes

- BODIPY staining is compatible with live cell imaging (cells can be imaged in medium)
- For live imaging, use FluoroBrite DMEM or phenol red-free medium to reduce background
- Staining can be combined with other fluorescent markers (use appropriate filter sets)
- For quantification, use image analysis software (ImageJ, CellProfiler) to measure droplet size and number
- Store BODIPY stock protected from light to prevent degradation