Lumiprobe
Life science solutions

Staining the Golgi Apparatus with BDP-Labeled Ceramides

Ceramides are precursors of sphingolipids composed of sphingosine and a fatty acid joined by an amide bond. BDP ceramides are synthetic fluorescent lipids, conjugates of BDP fluorophores with sphingosine. Inside the cell, BDP ceramides are incorporated into the membranes of the Golgi apparatus, so these stains are widely used in cell biology to visualize the Golgi apparatus in living and fixed cells with fluorescence microscopy.

Preparation of solutions

1.1 Stock solutions

BDP FL ceramide:

- Dissolve 50 µg BDP FL ceramide in 87.2 µL DMSO to obtain 1 mM of stock solution.
- Dissolve 250 µg BDP FL ceramide in 436 µL DMSO to obtain 1 mM of stock solution.

BDP TMR ceramide:

- Dissolve 50 µg BDP TMR ceramide in 73.6 µL DMSO to obtain 1 mM of stock solution.
- Dissolve 250 µg BDP TMR ceramide in 368 µL DMSO to obtain 1 mM of stock solution.

BDP TR ceramide:

- Dissolve 50 µg BDP TR ceramide in 70.8 µL DMSO to obtain 1 mM of stock solution.
- Dissolve 250 µg BDP TR ceramide in 354 µL DMSO to obtain 1 mM of stock solution.

Store the stock solutions at -20 °C or -80 °C away from light. Avoid repeated freeze-thaw cycles.

1.2 Staining solution

1. Measure 10 mL of Hanks' buffered salt solution with 10 mM HEPES (HBSS/HEPES), pH 7.4 into a 50 mL plastic tube. Other serum-free balanced salt solutions, such as PBS, are also suitable for this purpose.

   (Optional) Add 1 mM Ca^{2+} and 0.5 mM Mg^{2+} to the buffer solution to prevent living cells from rounding up and detaching from the glass.

2. Add 3.4 mg (0.34 mg/mL) of defatted BSA in the tube with buffer.
3. Add 50 µL of 1 mM ceramide stock solution to obtain 5 µM ceramide / 5 µM BSA working solution. The dilution of ceramides depends on the cell type and density and should be defined experimentally.

The resulting ceramide/BSA complex solution can be stored in plastic vials at -20 °C.

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Cell staining

2.1 Live cells

1. Grow cells on a sterile coverslip. Adherent cells can be stained directly on the coverslip.
2. Aspirate the medium from coverslip.
3. Rinse cells with an appropriate medium (such as HBSS/HEPES).
4. Incubate cells with 5 µM ceramide/BSA solution for 30 min at 4 °C.
5. Rinse cells several times with the ice-cold medium.
6. Rinse cells in a solution of defatted BSA (0.34 mg/ml) in HBSS/HEPES four times for 30 min at room temperature.
7. Incubate cells in fresh medium at 37 °C for a further 30 min.
8. Rinse cells in fresh medium.
9. *(Optional)* Stained cells can be fixed in 4% formaldehyde for 2 min at 4 °C. Wash fixed cells twice in PBS.
10. For fluorescent microscopy, invert the coverslip with stained cells onto the slide to place them between it and the coverslip.

2.2 Fixed cells

1. Fix cells in 4% formaldehyde for 5 min at 4 °C.
2. Wash fixed cells twice in PBS for 5 min each time.
3. Incubate cells with 5 µM ceramide/BSA in PBS for 30 min at 4 °C.
4. Rinse cells in a solution of defatted BSA (0.34 mg/ml) in PBS four times for 30 min at room temperature.
5. Rinse cells twice in PBS.
6. For fluorescent microscopy, mount cells under a coverslip using a mounting medium.

Spectral characteristics of BDP-labeled ceramides

	Absorbtion Max.*	Emission Max.*
BDP FL	503 nm	509 nm**
BDP TMR	542 nm	574 nm
BDP TR	589 nm	616 nm

*Absorption and fluorescence emission maxima determined in methanol. Values in labeled cells are similar.

**BDP FL-labeled ceramide also emits red fluorescence (~620 nm) in cells.

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