Cell receptor batch labeling with Tag-lite® SNAP-Lumi4®-Tb

PROTOCOL

Cells expressing the SNAP-tag receptor (ST-GPCR) can be labeled in batches with Tag-lite SNAP-Lumi4-Tb.

The following protocol describes the steps to label 20 million cells in T175 Flask (175cm2).

Use of different flasks requires different Tag-lite SNAP-Lumi4-Tb and Tag-lite labeling medium quantities listed at the end of the document (§ Cell culture containers:generalization).

Before labeling, an overnight incubation of the plated cells expressing the ST-GPCR is recommended for cell adhesion.

MATERIALS NEEDED FOR LABELING CELLS

- Tag-lite labeling medium Cisbio Ref# LABMED

- Tag-lite SNAP-Lumi4-Tb: fluorescent SNAP-tag substrate

	2 nmoles	5 nmoles	5 x 5 nmoles	100 nmoles
Cisbio Ref#	SSNPTBC	SSNPTBD	SSNPTBG	SSNPTBX

STORAGE AND HANDLING

- Upon reception, the Tag-lite labeling medium must be stored at 4°C until use
- Upon reception, the vial of SNAP Lumi4-Tb must be stored at -20°C until reconstitution.

Notes:

Once reconstituted with DMSO, the SNAP Lumi4-Tb must be used immediately or dispensed into disposable vials for storage at –80°C
DMSO solution may be frozen and thawed once.
DMSO solution is stable 6 months at -80°C

BATCH LABELING OF THE ADHERENT CELLS

1. Dilute 5 fold the Tag-lite labeling medium with distilled water in order to obtain a 1X working solution (sterile use)
2. Prepare the SNAP Lumi4-Tb

   - reconstitute the vial with 100% DMSO in order to obtain 100 µM stock solution, mix until completely dissolved
   - dilute the 100µM stock solution with Tag-lite labeling medium to obtain the working solution
3. Remove the cell culture medium from the T175 cell culture flask.
4. Onto the cells, gently add 10 mL of 100 nM Tag-lite SNAP-Lumi4-Tb diluted in Tag-lite labeling medium
5. Incubate the cells for 1h at 37°C + 5% CO2.
6. Gently wash the cells 4 times with 15mL of Tag-lite labeling medium.
7. Peel off, centrifuge and resuspend the cells in Tag-lite labeling medium

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8. Ensure that your cells are properly labeled by dispensing a small sample of your batch into a 384 well plate (20K cells per well) and a small sample of your unlabeled cells. Record the fluorescent signal at 620 nm. The Fluorescence recorded should be superior to fluorescence of the unlabeled cells dispensed
9. The cells may be used immediately or kept frozen at -80°C in freezing medium
    - If the cells are used immediately: plate the cells to carry out a binding assay
    - If the cells need to be frozen for later use: estimate the cell concentration by using standard counting methods, then, using standard freexing procedure, freeze cells at 1 to 2 million cells per vial

CELL CULTURE CONTAINERS: GENERALIZATION

The volume of Tag-lite labeling medium used to dilute the Tag-lite SNAP-Lumi4-Tb is chosen according to the surface of the container used to plate the cells. The volume of Tag-lite labeling medium is the one to clearly recover all the cells

	96 WELL PLATE	CELL CULTURE DISH 100 (59 CM^2)	CELL CULTURE DISH 150 (145 CM^2)	FLASK T175 (175 CM^2)
Volume of 100 nM SNAP-Lumi4-Tb	0.05 mL	4 mL	7.5 mL	10 mL
Volume of Tag-lite labeling medium used for each wasching step	0.1 mL	5 mL	10 mL	15 mL

FOR MORE INFORMATION
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