MANUAL

Technology: HTRF™ Tag-lite

Cell receptor batch labelling with Tag-lite™ SNAP-, CLIP- or Halo-Lumi4™-Tb

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For research only. Not for use in diagnostic procedures

Cells expressing the substrate-tag receptor (ST-GPCR) can be labelled in batches with Tag-lite substrate Lumi4-Tb. The following protocol describes the steps to label 20 million cells in T175 Flask (175cm^2).

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

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 Ref# LABMED

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

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

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

	20 nmoles	5 x 20 nmoles	500 nmoles
Revvity Ref #	SCLPTBE	SCLPTBF	SCLPTBZ

- Tag-lite HaloTag-Lumi4-Tb: fluorescent HaloTag substrate

	2 nmoles
Revvity Ref #	SHALOTBC

STORAGE AND HANDLING

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

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 ON 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 Lumi4-Tb subtrate
   - 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 Tag-lite substrate-Lumi4-Tb diluted in Tag-lite labeling medium used at:
   - 100 nM for Tag-lite SNAP-Lumi4-Tb
   - 1000 nM for CLIP-Lumi4-Tb
   - 100 nM for HaloTag-Lumi4-Tb

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

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 substrate-Lumi4-Tb is chosen depending on the surface of the container used to plate the cells. The volume of Tag-lite labeling medium is the amount necessary 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 substrate-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

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