



CELL TRANSIENT TRANSFECTION WITH TAGGED GPCR OR RTK PLASMIDS

PROTOCOL

Cisbio Bioassays proposes a broad list of GPCR and RTK plasmids encoding SNAP-tag®, CLIP-tag® and HaloTag® at the N-terminal position. Once transfected, cells expressing the tagged receptor can be labeled with the specific substrate derivatized with HTRF fluorophores.

MATERIAL

PLASMIDS FROM CISBIO

- The list of GPCR and TRK plasmids (human) is available on <http://www.cisbio.com/drug-discovery/tag-lite-gpcr-rtk-plasmids>

RECOMMENDED REAGENTS

- HEK 293 cells
- PBS (Life technologies Cat.# 10010-031)
- Cell dissociation buffer (Sigma Cat.#C5914)
- Opti-MEM, with GlutaMAX and phenol red (Life technologies Cat.# 51985-026)
- Lipofectamine 2000 (Life technologies Cat.#11668-027)
- Culture medium for HEK293: DMEM (Life technologies Cat.#31966-021) supplemented with Penicillin-Streptomycin 1X (Life technologies Cat.# 15070-063), HEPES 2mM (Life technologies Cat.#15630-122), MEM 1X (Life Technologies Cat.#11140-035)

TRANSIENT TRANSFECTION - FLASK T175

Two to three days before the transfection, seed the cells in a T175 flask. For an efficient transfection, cells should be adherent with a confluency around 70 - 80%

1. Prepare the transfection mix, add in the following order
 - Opti-NEM: 8 mL
 - Lipofectamine: 60 µL
 - Plasmid (1µg/µL): 20 µLIncubate for 20 minutes at RT
2. Gently remove the cell culture medium by aspiration
3. Wash cells with 5 mL of PBS
4. Add the 8.08 mL of transfection mix preparation then add 12 mL of cell culture medium
5. Incubate for 1 night at 37°C + 5% CO₂.
6. Cells expressing the substrate-tag receptor can now be labelled with Tag-lite SNAP-Lumi4-Tb and used to carry out a binding assay

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FOR MORE INFORMATION

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