

Seeding cells for LigandTracer®

This protocol describes the preparation of cell dishes with adherent cells to obtain one or several target areas and one reference area, suitable for measurements in LigandTracer.

Important information

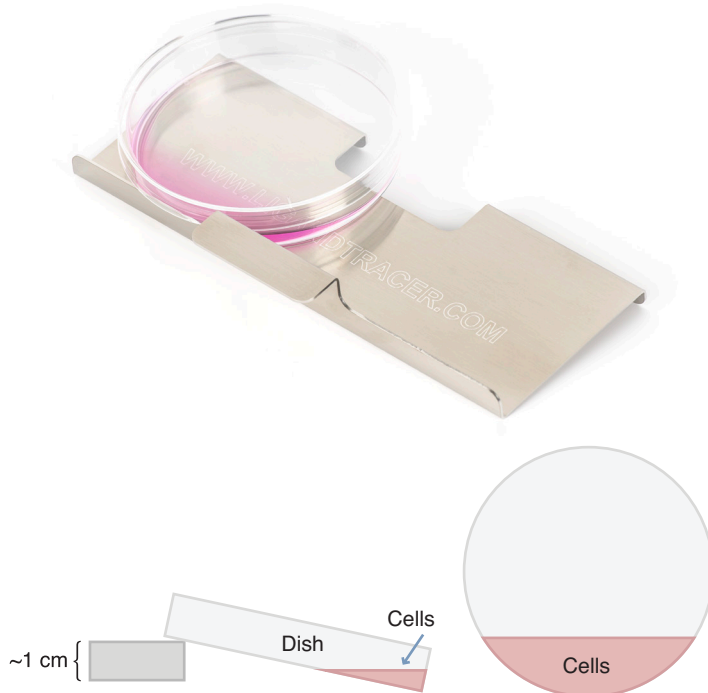
The cell dish should preferably be prepared at least one day prior to measurement.

Materials

- Cell culture dish, 87-89 mm in diameter and about 15 mm in height (e.g. Nunclon™ Cat. No. 150350)
- Adherent cells
- Cell culture media
- Trypsin or equivalent

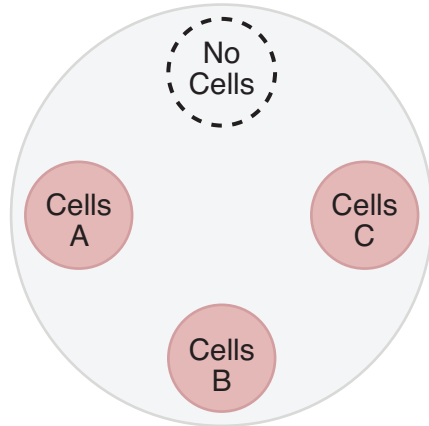
Procedure

1. Re-suspend the adherent cells in fresh solution using trypsin or an equivalent solution.
2. Seed cells in a 87-89 mm cell dish.
 - a. For one target area:
Tilt the empty cell culture dish by placing it on a LigandTracer cell dish holder or using a ~1 cm high object (e.g. a cell dish lid). Carefully dispense 2-3 ml of cell culture medium containing approximately one million cells into the lower part. Mark the lower end area with a pen so that you will easily find where the cells are seeded.



b. For several target areas:

Place the dish horizontally and add cells as 0.5-1 ml droplets with approximately 10^6 cells/ml near the rim of the dish. Keep one area cell free, to be used as a reference.



3. Place the dish in an incubator (still tilted if having one target area) and let the cells attach firmly. This typically takes 3-6 hours, but is dependent on the cell line.
4. Carefully remove the remaining cell solution once the cells have attached. Place the dish horizontally and add approximately 10 ml cell medium. Leave the dish in the incubator until measurement in LigandTracer, preferably at least over-night.
5. Confirm under a microscope that the cells have attached only where intended. Remove with a cell scraper if you find more than a few scattered cells in areas far from where the cells were seeded.
6. Make sure to use instrument settings that are suitable for the number of cell areas that you have prepared. Choose any of the pre-defined templates in LigandTracer Control or define your own settings by unlocking an opened template.