LigandTracer

Protocol

Seeding cells in LigandTracer cell culture MultiDish 2×2

This protocol describes the preparation of LigandTracer **cell culture** MultiDish 2×2 with adherent cells to obtain one target area and one reference area in each compartment, which is suitable for measurements in LigandTracer Green.

Important information

The cell dish should preferably be prepared at least one day prior to the LigandTracer experiment. It is highly recommended to perform cell culture work in a sterile environment.

Materials

- LigandTracer *cell culture* MultiDish 2×2* (Cat. No. 1-4-201, Ridgeview Instruments AB)
- Adherent cells
- Cell culture medium
- Trypsin or equivalent

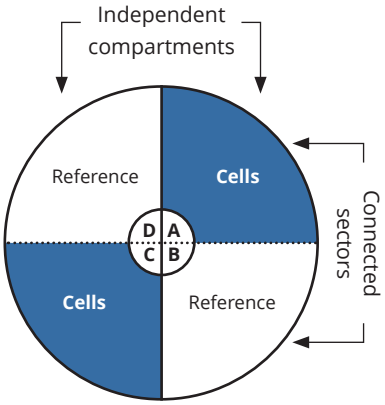
Procedure

1. Re-suspend the adherent cells in fresh cell culture medium using trypsin or an equivalent solution.
2. Carefully dispense 1.5 ml cell suspension into each of the sectors A and C. Ensure that the cell suspension is distributed over the entire quarter. Aim at having a confluent cell layer at the time of the LigandTracer experiment, assuming that it does not affect the viability of your cell line. For example, one to two million cells in each quarter may be suitable for measurements that start the next day. It is also possible to add cells as 0.5-1 ml droplets with approximately one million cells/ml in the sections A and C. Keep sections B and D as cell free reference sectors.
3. Place the dish in an incubator and let the cells attach firmly. This typically takes 3-6 hours depending on the cell line.
4. Carefully remove the remaining cell solution once the cells have attached. Add approximately 5 ml cell medium to each compartment (each half). It is advised to work with medium that contains fetal bovine serum or a similar protein cocktail to reduce the risk of non-specific ligand binding during the measurement.
5. Keep the dish in the incubator until the LigandTracer experiment, preferably at least over-night.
6. 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 the reference areas. Replace media with 1.8 ml fresh medium in each compartment (each half).
7. Choose a pre-defined MultiDish 2x2 template in LigandTracer Control that provides suitable settings for the experiment, and start the measurement. The detection time can be altered by unlocking the template.

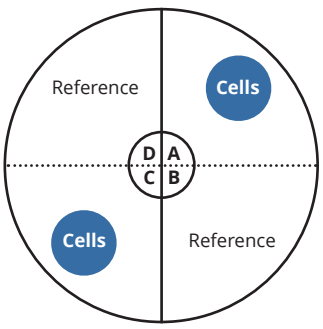
More information can be found in protocol *A typical LigandTracer measurement with MultiDish 2x2*.

* LigandTracer MultiDish 2x2 is only compatible with LigandTracer Green Second generation (serial numbers RCF-041XXX) or older LigandTracer Green instruments that have been upgraded.

Seeding in quarters



Seeding in droplets



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