LigandTracer

Protocol

A typical LigandTracer measurement with MultiDish 2x2

This protocol describes a general LigandTracer Green assay to study the affinity and the association and dissociation rates of interactions, using MultiDish 2×2 with one target area and one reference area in each compartment.

Materials

- LigandTracer *cell culture* MultiDish 2×2* (Cat. No. 1-4-201, Ridgeview Instruments AB) containing approximately 1 million cells in a local area (please refer to the protocol *Seeding cells in cell culture MultiDish*) and at least 10^{11} targets in total
- Labeled ligand in a stock solution
- Cell culture media
- LigandTracer control software version 2.3 or higher

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

Procedure

1. Replace the media of the prepared MultiDish 2x2 with 1.8 ml fresh cell culture media in each compartment (each half).

2. Start *LigandTracer Control* through the shortcut found on the desktop of the computer. A connection between computer and instrument is established.

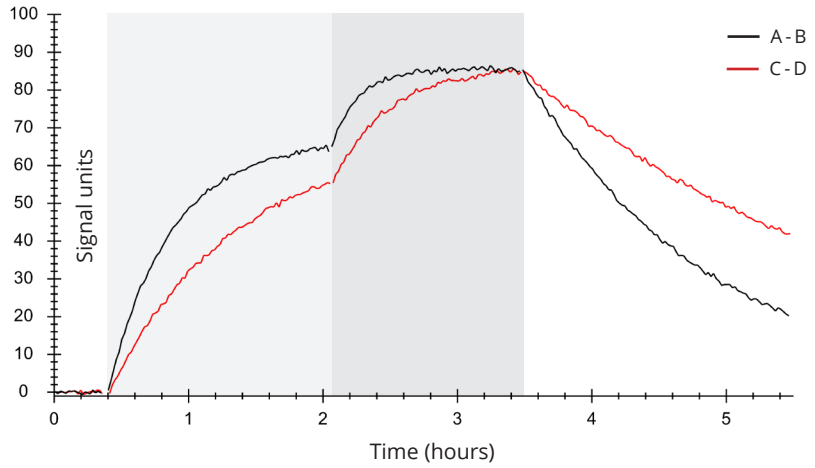
3. Suggested assay templates are listed in the *General assay* area of the start view. Click on the *2x2 standard* button under *MultiDish Assay*. It is possible to edit the name of each detection position of the dish (e.g. the name of the cell line) in the list below the figure. This information is visible during the experiment and saved in the result file. It is also possible to enter information about the experiment, such as project name or cell treatment in the *Experiment information* section to the right of the screen.

4. Remove the lid from the cell dish and place the dish in the LigandTracer instrument. Make sure that the cells are at the target positions (*T*) as presented in the dish overview image of the software and that there are no visible bubbles in the dish.

5. Close the instrument lid, press *Start* and give the file a suitable name. The measurement starts and two curves representing signal (y axis) vs. time (x axis) are displayed in the graph. These are reference subtracted curves, meaning that it depicts the signal from the target position subtracted with the signal from the background position. This phase is called the Baseline measurement and shows the signal when no labeled ligand has been added yet. Continue the Baseline measurement for 15-30 minutes to ensure that the baseline signal is stable.

6. Open the *Experiment planner* (*Tools* menu) and provide information on your experimental steps. You can abbreviate p for pM, n for nM, u for µM and m for mM. As a starting point, a concentration close to the expected affinity (K_D) is recommended.

Example of LigandTracer data, different ligand in A/B and C/D



7. Press *Pause* and wait till the current rotation finishes and a side panel appears next to the graph. Information from the *Experimental planner* can be transferred to the side panel in one click by pressing *Send* for the chosen phase. Alternatively, you can select *Association* as the purpose of the next phase in the side panel and enter the ligand name and its final dish concentration. Ligand names and concentrations are saved in the result file and used during data evaluation with *TraceDrawer* (version 1.9 or higher).

8. Open the LigandTracer lid, add the labeled ligand in both compartments, close the lid and press *Continue*. It is possible to rotate the black cell dish holder by hand before adding or removing ligand and medium. In this way, one avoids exposure of cells to turbulent flow and/or uneven ligand concentrations during ligand addition. The cell dish holder is automatically rotated to its home position when resuming the experiment. Please do not move/remove the dish from the cell dish holder during the experiment.

9. The signal will increase if the ligand binds to the target on the cells. Continue the measurement until a clear curvature is observed or until equilibrium has been reached (visible as a signal plateau). Press *Pause* and wait for the side panel to appear.

10. Add additional ligand to increase the concentration approximately threefold. Transfer the ligand concentration from the *Experimental planner* or enter the new ligand concentration in the side panel and press *Continue*. By including at least two ligand concentrations in one measurement, more and/or more accurate information about the interaction is obtained. Repeat step 9.

11. Replace the ligand solution with fresh cell culture medium. Choose *Dissociation* as the purpose of the next phase. Restart the experiment to measure the dissociation rate of the ligand, preferably until the signal decreased with at least 15 %. You can leave the dissociation measurement unattended and remove redundant data during evaluation.

12. To finish the measurement, pause the experiment and press *End* on the side panel.