LigandTracer

Protocol

# A typical LigandTracer measurement

This protocol describes a general LigandTracer assay to study the affinity and the association and dissociation rates of an interaction, using one target and one reference area in a Petri dish.

## Materials

- Cell culture Petri dish, 87-89 mm in diameter and about 15 mm in height, containing approximately 1 million cells in a local area (please refer to the protocol *Seeding cells in LigandTracer cell culture MultiDish 2x2*), with at least $10^{11}$ targets in total
- Labeled ligand in a stock solution
- Cell culture media
- LigandTracer control software version 2.3 or higher

## Procedure

1. Replace the media of the prepared Petri dish[1] with 3 ml fresh cell culture media.

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 *One target* button. 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.

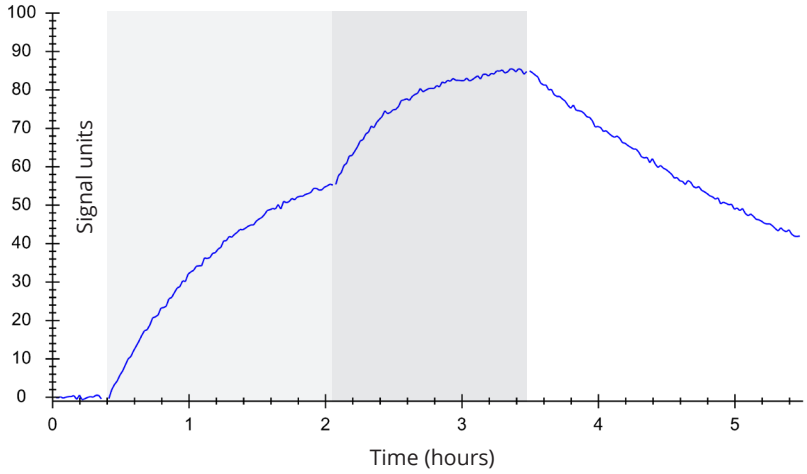
4. Remove the lid from the cell dish and place the dish in the LigandTracer instrument. Make sure that the cells are at the target position (*T*) as presented in the dish overview image of the software, i.e. opposite the single steel pin. For LigandTracer Green, make sure that there are no visible bubbles in the dish.

5. Close the instrument lid, press *Start* and give the file a suitable name. The measurement starts and a curve representing signal (y axis) vs. time (x axis) is displayed in the graph. This is a reference subtracted curve, meaning that it depicts the signal from the target position that is 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 (LigandTracer Green) or a few minutes (LigandTracer Yellow, LigandTracer Grey, LigandTracer White) 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 starting point, a concentration close to the expected affinity ($K_D$) is recommended.

[1] See protocol *Seeding cells for LigandTracer*

Example of LigandTracer data




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 and higher).

8. Open the LigandTracer lid, add the labeled ligand, 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 unequilibrated ligand concentrations during ligand addition. The cell dish holder is automatically turned 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 three-fold. 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 on 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 run to measure the dissociation rate of the ligand, preferably until the signal decreased at least with 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* in the side panel.

**Note:**
A more detailed description can be found in the *LigandTracer® Control and Application Handbook*.