

# A typical LigandTracer® measurement

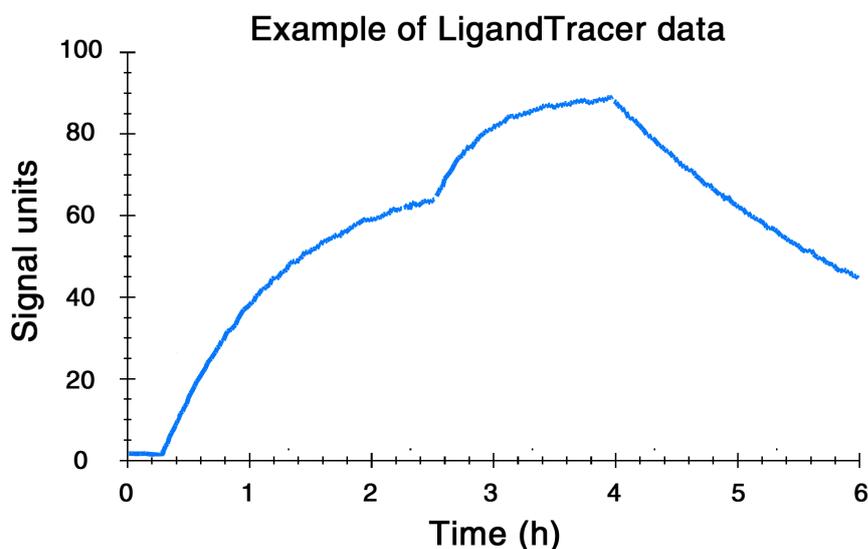
This protocol describes a general LigandTracer assay to study the association and dissociation rates of an interaction, using one target area. A more detailed description can be found in the LigandTracer® Control and Application Handbook.

## Materials

- Cell 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 for LigandTracer”), with at least  $10^{11}$  targets in total
- Labeled ligand in a stock solution
- Cell culture media

## Procedure

1. Replace the media of the prepared LigandTracer dish 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. A list of suggested assay templates are listed in the General assay area of the start view. Click on the One target button.
4. Remove the lid from the cell dish and put the dish in LigandTracer. 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. 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 run and saved in the result file.
5. Press Start run 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 height 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 yet been added. Continue the Baseline measurement for 15-30 minutes (LigandTracer Green) or a few minutes (LigandTracer Yellow, LigandTracer Grey, LigandTracer White) to ensure a stable system.



6. Press Pause Run. A side panel appears next to the graph once the current rotation has finished and the detection has paused, which may take up to one minute. *Do not open the lid before the side panel has appeared.* Select Association as the purpose of the next phase and enter the ligand name and its final dish concentration, which will be saved in the result file. Abbreviate p for pM, n for nM, u for  $\mu$ M and m for mM. A concentration close to the  $K_D$  value is recommended, if possible.
7. Open the LigandTracer lid, add the labeled ligand, close the lid and press Continue run.
8. The signal will eventually start to 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 Run and wait for the side panel to appear.
9. Add additional ligand to increase the concentration approximately three times. Enter the new ligand concentration and press Continue Run. Including at least two ligand concentrations in one measurement will add more information about the interaction. Repeat step 8.
10. 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 at least 15 % of the signal has decreased.
11. To finish the measurement, pause the run and press End run on the side panel.