

Multiple cell line assay in a single LigandTracer® run

LigandTracer instruments detect binding of labeled ligands to cells in real-time. Often, one cell colony per dish is investigated. This application note describes an alternative approach with two or three cell colonies in one dish, enabling comparisons between different cell lines or clones.

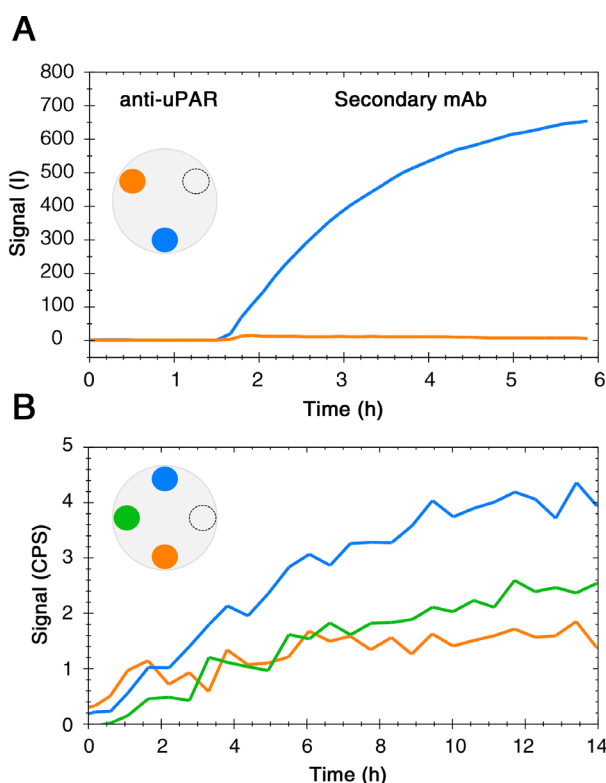
Experiment details

Specificity measurement of anti-uPAR

A cell culture dish containing a urokinase receptor (uPAR) expressing cell line (Fig. A, blue), a uPAR negative cell line (Fig. A, orange) and a cell free reference area (Fig. A, empty circle)² was incubated with un-labeled anti-uPAR antibody for 90 min in LigandTracer Green³, followed by incubation of an Alexa Fluor 488-labeled secondary mAb. A clear signal was observed from the positive cells but not from the negative cells, indicating a specific binding of anti-uPAR to uPAR.⁴

Comparison of transfection levels of a GPCR

Binding of ¹²⁵I-labeled porcine peptide YY (pPYY) to wild type (wt) HEK-293 cells (Fig. B, orange) or HEK-293 cells with a stable (Fig. B, blue) or transient expression (Fig. B, green) of the GPCR human Y2 receptor (hY2R) was studied in parallel in LigandTracer Grey^{2,3}. The stable cell line produced the highest signal and the wt the lowest. Additionally, the signal from the transfected cell lines continued to increase over time, while the wt HEK-293 signal remained stable.¹



Conclusions

Comparing the binding of a labeled ligand to several cell colonies in one run is beneficial when studying the specificity of an interaction and to understand differences between strains and clones. Results show, in both cases, that ligand binds specifically to target expressing cells and that the signal is easily distinguishable between different cell areas.

Reference and protocols

1. Xu B, et. al. *Detecting ligand interactions with G protein-coupled receptors in real-time on living cells*. *Biochem Biophys Res Commun*. 2013. 441(4):820-824.
2. Protocol: Seeding Cells for LigandTracer®
3. Protocol: A typical LigandTracer measurement
4. Ridgeview Instruments AB thanks Dr Anna-Karin Olsson and Dr Staffan Johansson at Dept. of Medical Biochemistry and Microbiology, Uppsala University, for providing access to their uPAR results.

Protocols can be downloaded at www.ridgeview.eu/download/