

LigandTracer MultiDish: Increasing throughput and simplifying comparison

The LigandTracer MultiDish not only saves working time and doubles the throughput of your LigandTracer instrument, but also facilitates comparative studies. In this application note, we describe the use of LigandTracer *cell culture* MultiDish 2×2 to study competition and proximity.

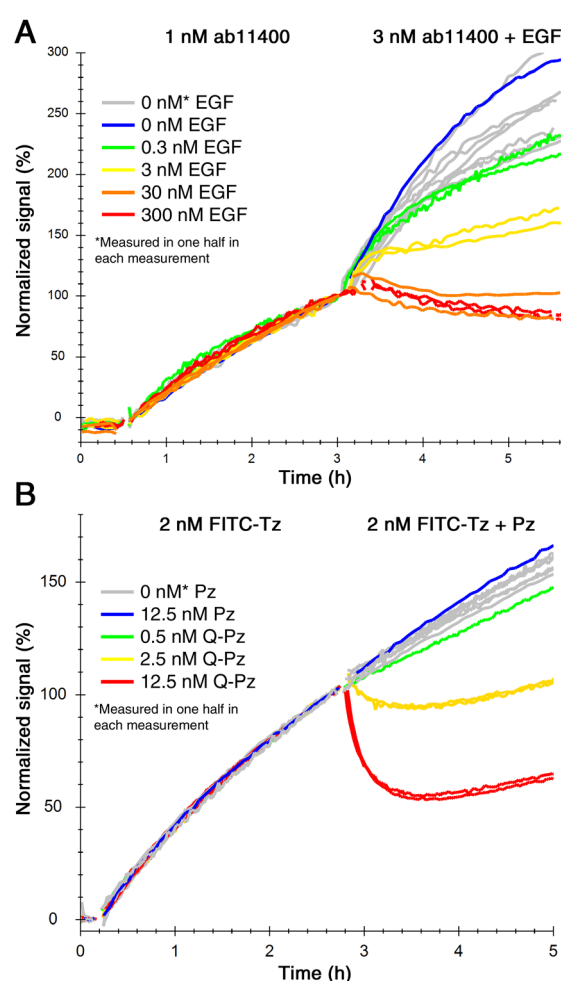
Experiment details

EGFR competition

A FITC-labeled antibody targeting the epidermal growth factor receptor, EGFR (Cat. No. ab11400, Abcam), was added stepwise to A431 cells. Upon increasing the ab11400 concentration to 3 nM, human epidermal growth factor (hEGF) was added to one half of the LigandTracer *cell culture* MultiDish 2×2, while the other half acted as a positive control without hEGF (Fig. A)^{1,2}. The signal decreased in a concentration dependent manner in presence of the unlabeled hEGF. This concentration dependent competition demonstrates that the antibody is specific and that hEGF either has an overlapping epitope or an allosteric effect on binding of the antibody.

Trastuzumab/pertuzumab proximity assay

Binding of 2 nM FITC-labeled anti-HER2 antibody trastuzumab (FITC-Tz) to SKOV3 cells was measured in both halves of a LigandTracer *cell culture* MultiDish 2×2 (Fig. B – grey curves as controls)^{1,2,3}. After 2.5 h incubation, the quencher-labeled anti-HER2 antibody pertuzumab (Q-Pz) was added to one half, resulting in concentrations of 0.5-12.5 nM (green, yellow, red). The antibodies were not competing, as illustrated by the use of 12.5 nM unlabeled Pz in one experiment (blue). The decrease in signal when adding quenching pertuzumab indicates close proximity of the antibodies, suggesting binding to the same receptor.



Conclusions

With the LigandTracer MultiDish you can obtain reproducible data and compare ligand binding to cells that are cultured, handled and measured at identical conditions. Investigate the difference between cell lines, use different treatments, introduce a competitor or add a quencher – comparative interaction studies on living cells have never been this easy!

Reference and protocols

1. Protocol: Seeding cells in LigandTracer *cell culture* MultiDish
2. Protocol: A typical LigandTracer measurement
3. Protocol: Protein labeling with FITC

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