



Fluorescence detection with LigandTracer® Green

TECHNOLOGY NOTE

LigandTracer® Green monitors biomolecular interactions on cells in real-time using fluorescently labeled biomolecules. Measurements of e.g. on-off rate, affinity or retention can be made using blue (~488nm) and green (~535nm) fluorophores, e.g. FITC, Alexa Fluor® 488, as labels. This technology note describes the uptake and retention of Alexa Fluor 488-labeled Cetuximab to A431 cells.

Experimental

Cancer cells of type A431 (human epithelial carcinoma cell line) were seeded in a local area of a cell dish and were allowed to attach firmly to the surface for at least 24 hours. The EGFR-binding antibody Cetuximab was labeled with Alexa Fluor 488 according to protocols from the manufacturer. The cell dish with 3 ml cell culture medium (HAMS-F10) was placed in a LigandTracer Green prototype, schematically illustrated in figure 1. The difference between the fluorescence intensity of the target cell area and the area opposite to the target cells was measured every minute by rotating the dish. After a short baseline measurement, Cetuximab was added (4.4 nM) and the uptake was detected automatically for 2 h. The instrument was stopped and the medium containing antibody was replaced with fresh medium, followed by a retention measurement.

For comparison, the interaction of ¹²⁵I-labeled Cetuximab (4.4 nM) binding to A431 cells was detected using LigandTracer Grey. The accuracy of LigandTracer Grey has previously been proven.

Results

Results from the interaction measurements for Alexa Fluor 488-Cetuximab and ¹²⁵I-Cetuximab are shown in Figure 2. The curvature of the uptake and retention from the two runs are basically the same, verifying that LigandTracer Green can produce accurate real-time binding traces.

Conclusions

LigandTracer Green offers a simple and accurate method for real-time measurement of biomolecular interactions to cells. Binding data of fluorescently labeled proteins is obtained during minutes to hours. LigandTracer Green reproduces results made with a validated radioactivity instrument, with similar sensitivity.

References

Application notes and references are available at www.ligandtracer.com

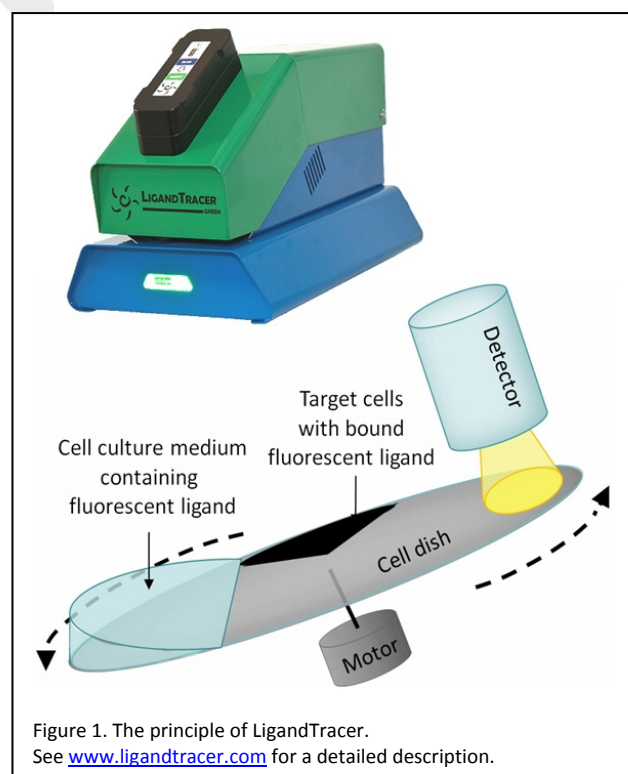


Figure 1. The principle of LigandTracer. See www.ligandtracer.com for a detailed description.

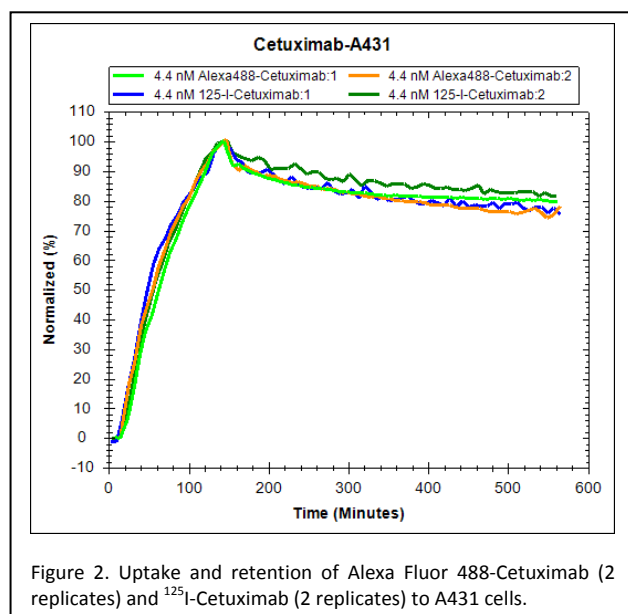


Figure 2. Uptake and retention of Alexa Fluor 488-Cetuximab (2 replicates) and ¹²⁵I-Cetuximab (2 replicates) to A431 cells.