



# Cell comparison in a single LigandTracer® run

## TECHNOLOGY NOTE

LigandTracer® instruments measure the binding of labeled ligands to cells in real-time, normally using one cell colony per dish and run. This is a straightforward approach with data easy to evaluate. This technology note describes an alternative to this, using two cell colonies in one dish, enabling a comparison between different cell strains or clones. The method is also applicable to analysis of multiple tissue samples.

## Experimental

In brief, two separate cell colonies were seeded in a single circular cell dish, as shown in Figure 1. This means that the fluorescently labeled ligand is incubated with the two cell colonies simultaneously in LigandTracer Green. For tissue analysis, three different tissue samples (one positive control, one negative control and one xenograft subject to analysis) were placed evenly spread in the cell dish, and the interaction of a <sup>125</sup>I-labeled polyclonal anti-HER2 antibody (DAKO) was measured in LigandTracer Grey. Experimental details are found on next page.

## Results

The signal from Alexa Fluor® 488-labeled mAb binding to an anti-uPAR-mAb is found in figure 2. The result show that the anti-uPAR-mAb clearly binds to the uPAR-expressing cells A but not to cell B, lacking the receptor. The results indicate a specific interaction, as no binding is observed to the cells used as a negative control. The insignificant signal from the negative control also shows that the measurement on cell B area is not affected by the high signals from the cell A area. The same pattern is seen for tissue analysis: The positive control and the xenograft both give clear binding signal, while as the negative control does not (Figure 3).

## Conclusions

Comparing the binding of a labeled ligand to at least two cell colonies in one run is possible. This is a method to study the specificity of an interaction and to understand differences between strains, clones, different tissue samples... The results show, in both cases, that the antibody binds specifically to the positive control and that the signal is easily distinguished between the different cell/tissue areas.

## References

Ridgeview Instruments AB thanks Dr Anna-Karin Olsson and Dr Staffan Johansson at Dept. of Medical Biochemistry and Microbiology at BMC, Uppsala, for providing access to their uPAR results. Tissue measurements were conducted at Ridgeview Instruments AB. For further information, visit [www.ligandtracer.com](http://www.ligandtracer.com).

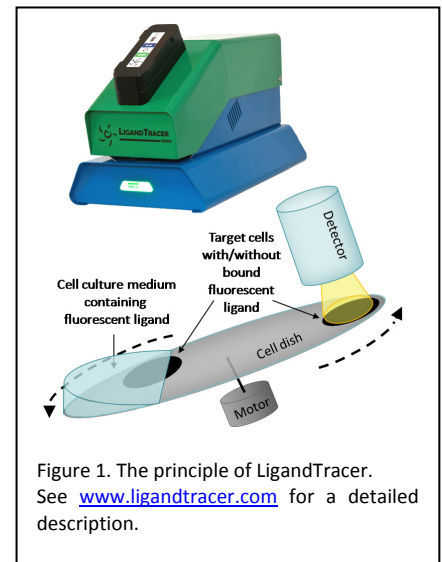


Figure 1. The principle of LigandTracer. See [www.ligandtracer.com](http://www.ligandtracer.com) for a detailed description.

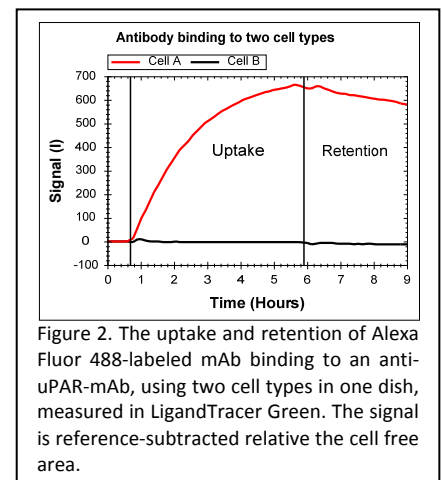


Figure 2. The uptake and retention of Alexa Fluor 488-labeled mAb binding to an anti-uPAR-mAb, using two cell types in one dish, measured in LigandTracer Green. The signal is reference-subtracted relative the cell free area.

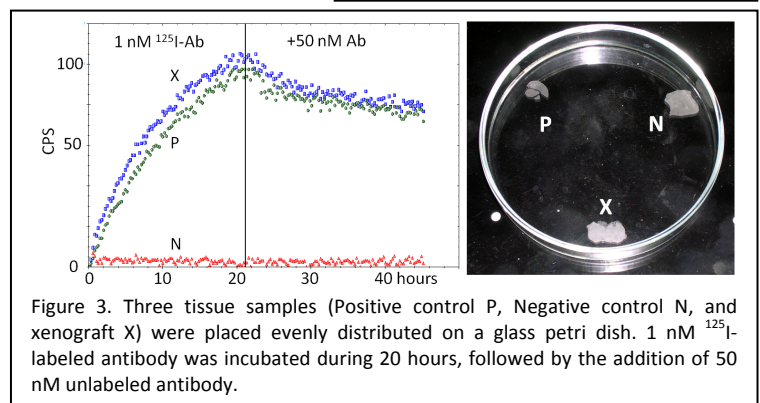


Figure 3. Three tissue samples (Positive control P, Negative control N, and xenograft X) were placed evenly distributed on a glass petri dish. 1 nM <sup>125</sup>I-labeled antibody was incubated during 20 hours, followed by the addition of 50 nM unlabeled antibody.



## Experimental

Small volumes of cell containing medium was added to local parts of a circular cell dish, spread spatially according to figure 4 to maximize the separation of the colonies from each other and from the cell free area used as a reference. The cell suspensions were added near but not in contact with the rim of the dish. The cell suspension droplet stayed in position due to the surface tension. The cells were allowed to attach firmly to the dish prior to addition of fresh medium. The dish was left in incubator over night to thoroughly coat all plastic surfaces with the protein cocktail of the medium.

An unlabeled antibody targeting the receptor uPAR was incubated for approximately 90 min in LigandTracer Green. Cell-line A expresses the receptor uPAR while as cell-line B does not, hence making cell-line B a suitable negative control. The antibody containing medium was removed and new medium containing an Alexa Fluor 488-labeled secondary mAb was added. The binding of the secondary antibody to anti-uPAR-mAb was monitored. LigandTracer was set to detect 20 positions around the rim of the dish (numbered 0-19 counter-clockwise, see fig 5). Thus, the positions of interest were approximately positions 2 (cell-line B), 9 (cell-line A) and 15 (reference area, no cells). In the evaluation part of the software, the peak and background was defined to study the reference subtracted signal from cell-line A (peak: 9, bg: 15), cell-line B (peak: 2, bg: 15) and the difference between A and B (peak: 9, bg: 2).

In tissue analysis, the tissue samples were placed evenly distributed as indicated in Figure 3. The procedure of attaching tissue samples to a glass petri dish is described in more detail in the application note *Monitoring protein-tissue interactions in real-time* and in the scientific publication *Real-time immunohistochemistry analysis of embedded tissue*<sup>1</sup>.

## Alterations of the method

In order to increase the time resolution of the measurement, 10 stops per round can be chosen instead of 20. The areas of interest are then found on approximately positions 1 (cell-line B), 4 (cell-line A) and 7 (no cells) if the dish is positioned according to figure 4.

Three cell colonies can be run in one dish: either replace the cell free area with the third colony, or split the cell dish into four areas. When using LigandTracer Grey or Yellow, the dish should not be split in more than three areas to maintain an adequate separation of the signals.

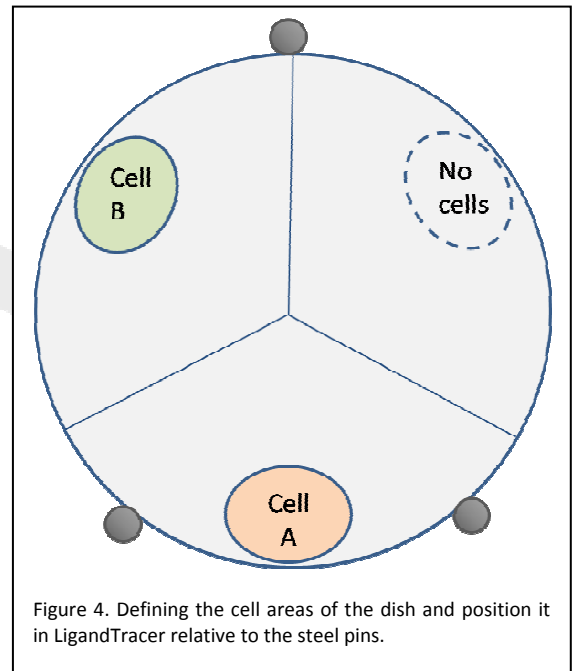


Figure 4. Defining the cell areas of the dish and position it in LigandTracer relative to the steel pins.

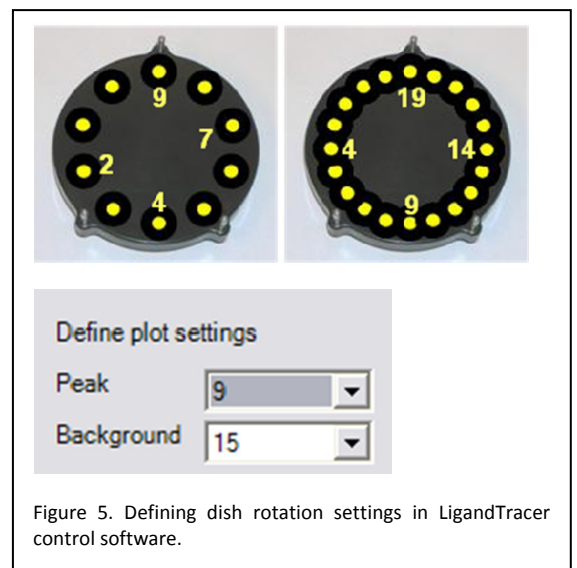


Figure 5. Defining dish rotation settings in LigandTracer control software.

<sup>1</sup> Lars Gedda, Hanna Björkelund, and Karl Andersson, *Real-time immunohistochemistry analysis of embedded tissue*, Applied Radiation and Isotopes, 2010 <http://dx.doi.org/10.1016/j.apradiso.2010.06.003>