



# Monitoring protein-tissue interactions in real-time

## APPLICATION NOTE

The analysis of how proteins interact with tissue is currently performed using immunohistochemistry (IHC), a semiquantitative binding assay which relies on staining of antibodies specific for selected receptors. In this application note we show how LigandTracer can be used for real-time detection of how antibodies (Abs) interact with tissue, both as labelled primary Abs and by use of secondary Abs.

### Experimental

In a first example, a paraffin-embedded SKOV-3 xenograft tumour from a mouse was placed on a pretreated glass petri dish, was de-paraffinized and heat-treated for epitope retrieval (Fig 1). The glass dish was placed in LigandTracer Grey and an anti-HER2 Ab (DAKO, Glostrup, Denmark) labelled with  $^{125}\text{I}$  was added (1 nM). After 20 hours of incubation, unlabelled anti-HER2 Ab was added at a total concentration of 10 nM.

In a second example, paraffin-embedded tissue from three different sources (breast from a breast cancer patient, tonsil from a healthy subject, and placenta from a healthy subject) was (i) placed on a pretreated glass dish, (ii) de-paraffinized, (iii) heat-treated for epitope retrieval, and (iv) incubated with a primary anti-HER2 Ab at 1 nM (DAKO) for one hour. After thorough wash, the glass dish was positioned in a LigandTracer Green prototype at room temperature and FITC-labelled secondary Ab directed against the primary Ab was added to a final concentration of 40 nM. A consecutive tissue section was stained according to standard IHC protocols for comparison.

### Results

Figure 2 shows the primary interaction of radiolabelled anti-HER2 Ab (DAKO) to SKOV-3 xenograft. There is a clear binding phase during incubation and it takes more than 20 hours to approach equilibrium for this interaction at 1 nM of the Ab. Upon addition of unlabelled Ab, the signal level decreases as a result of competition for the binding sites. This decrease is a strong indication of a specific interaction.

Figure 3A shows positive IHC-staining of breast cancer, intermediate staining of placenta and negative staining for tonsil. Figure 3B shows how the secondary Ab binds to the tissue sections in the glass dishes. When incubation media is replaced with buffer the beginning of washout is seen. The real-time interaction pattern after incubation with secondary Ab is similar to the IHC staining results.

### Conclusion

Real-time interaction analysis of how antibodies bind to tissue is made possible – both for primary and secondary antibody interactions.

### References

A technology note describing LigandTracer in general can be downloaded at [www.ligandtracer.com](http://www.ligandtracer.com). Ridgeview Instruments AB thanks Dr Lars Gedda and Dr Kenneth Wester at Uppsala University, Sweden, for generously sharing his results.

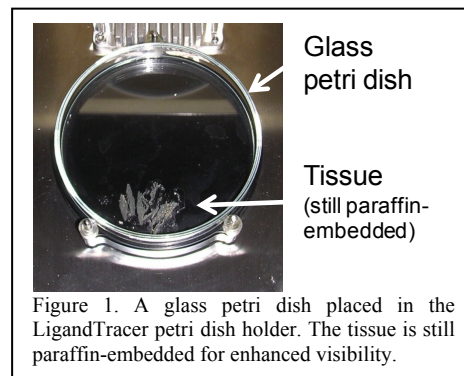


Figure 1. A glass petri dish placed in the LigandTracer petri dish holder. The tissue is still paraffin-embedded for enhanced visibility.

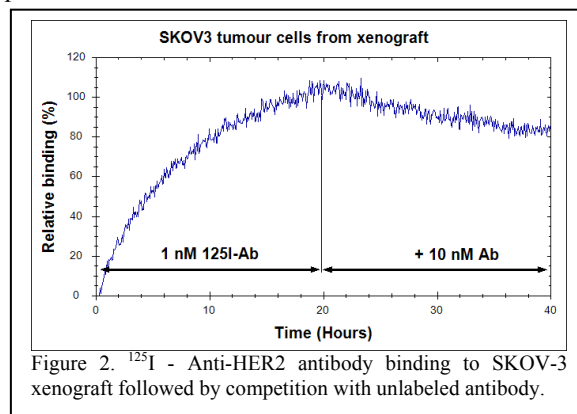


Figure 2.  $^{125}\text{I}$  - Anti-HER2 antibody binding to SKOV-3 xenograft followed by competition with unlabeled antibody.

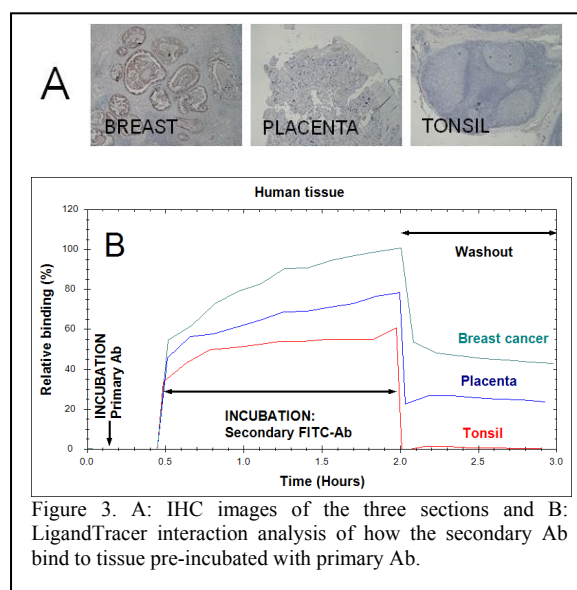


Figure 3. A: IHC images of the three sections and B: LigandTracer interaction analysis of how the secondary Ab bind to tissue pre-incubated with primary Ab.