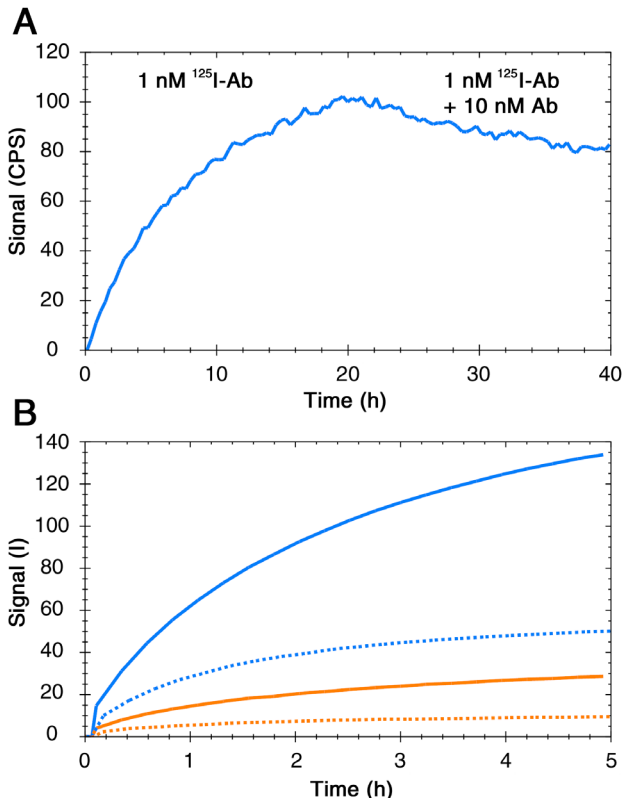
Monitoring protein-tissue interactions in real-time

The analysis of how proteins interact with tissue is currently performed using immunohistochemistry (IHC), a semiquantitative binding assay that relies on staining of antibodies specific for selected receptors. This application note demonstrates how LigandTracer® can be used to investigate how antibodies (Abs) interact with tissue, using either a labeled primary or a secondary Ab, through time-resolved measurements.

Experiment details

Specific binding of anti-HER2 to SKOV-3 xenograft tumor tissues

The interaction between 1 nM ^{125}I-labeled anti-HER2 Ab (A0485, DAKO, Glostrup, Denmark) and paraffin-embedded SKOV-3 xenograft tumour tissue from mouse was monitored with LigandTracer Grey (Fig. A).[3,4] The interaction was slow and required more than 20 hours to approach equilibrium. After 20 hours of incubation, un-labeled anti-HER2 Ab was added to obtain a concentration of 10 nM. The competition for binding sites caused a signal decrease, indicating specificity of the interaction.[1]



Using LigandTracer to optimize your IHC protocol

The effect of varying the primary antibody pre-incubation time prior to measurement was studied in LigandTracer Green, using unlabeled anti-RBM3 Ab and tissue from nasopharynx (Fig. B, blue) and tonsil (Fig. B, orange).[3,4] The signals after 1 hour pre-incubation were lower than those obtained after 3 hours in both systems (Fig B, dotted lines and solid lines respectively) indicating that incubation times >1 hour would benefit IHC, with the prospect of a high and clear staining at 3 hours. The secondary antibody required more than 5 hours to reach equilibrium at assay concentration (6.7 nM), relevant to consider because end-point methods such as IHC require measurement at equilibrium to achieve highest robustness. In agreement with the IHC results, the signal from the tonsil tissue was low in comparison to signal from the nasopharynx one, validating LigandTracer as an optimization tool for IHC.

Conclusions

Real-time interaction analysis of how antibodies bind to tissue is made possible with LigandTracer – both for primary and secondary antibody interactions. Following interactions over time enables a better understanding of the different processes applied in conventional IHC, leading to optimized assay protocols with improved sensitivity.

Reference and protocols

1. Gedda L, et. al. *Real-time immunohistochemistry analysis of embedded tissue.* 2010. 68(12):2372-2376.
2. Dubois L, et. al. *Evaluating real-time immunohistochemistry on multiple tissue samples, multiple targets and multiple antibody labeling methods.* BMC Res Notes. 2013. 6(1):542.
3. Protocol: A typical LigandTracer® measurement
4. Protocol: Preparations for tissue measurements in LigandTracer®

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