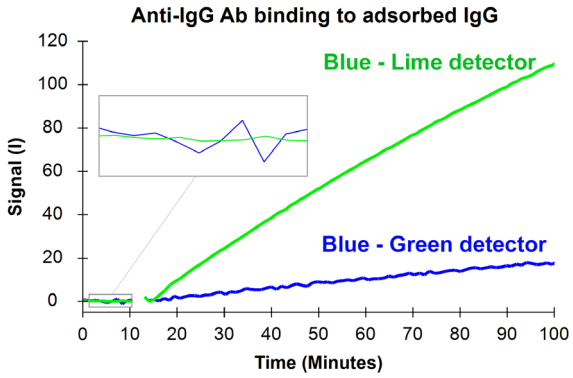
Technology Note

Blue-Lime detector

Label your ligand with the bright fluorescent protein Phycoerythrin (PE) and measure binding with the Blue-Lime detector of LigandTracer® Green to obtain stable signals with minimal noise.

Blue-Lime detector technology

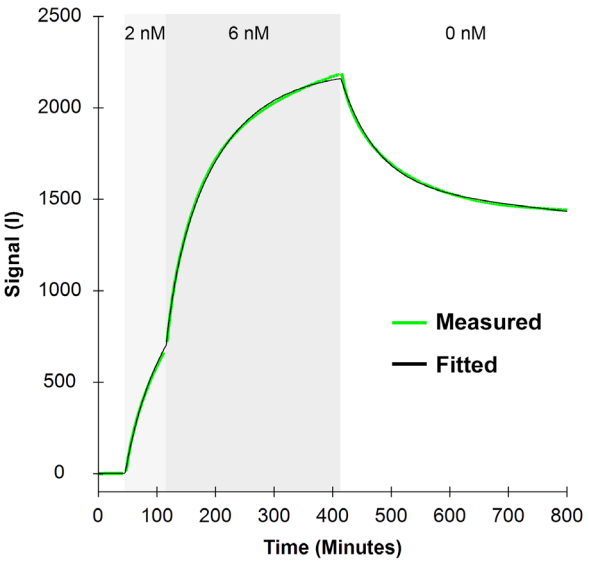
LigandTracer Green includes an interchangeable detector that broadens its use. Easily swap the detectors without any tools needed. PE is a bright protein-based fluorophore with a relatively large shift between its excitation and emission wavelengths. The Blue-Lime detector matches this large difference (ex: 488 nm, em: 575 nm), resulting in minimal interference from the light source and thus low noise levels.



Application Example

Materials and methods

CHO-X cells, induced to express the target EpCAM (CD326) or the irrelevant target PD1 (negative control), were tethered to a MultiDish 2×2 *for coating* according to the BAM protocol[1,2] and kept in medium over-night to recover target expression. Prior to measurement, an anti-EpCAM IgG (aEpCAM) was indirectly labeled by incubating with equimolar concentrations of a PE-labeled anti-human IgG Fc antibody (PE-Ab) (BioLegend UK, #409304). Measurement of binding of 2 and 6 nM of PE-Ab/aEpCAM was followed by a dissociation phase in regular medium[3]. This example was kindly provided by Shalom Gurjat at Oxgene, UK.



Results

The bright PE fluorophore created signal-to-noise levels exceeding 2000 and a signal drift below 2 signal units per hour during baseline measurement. The OneToTwo model (TraceDrawer) fitted the data well, presenting two interactions with a 17-fold difference in affinity (2.2 nM and 0.13 nM) that primarily differed in their binding stability, as reflected by the dissociation rate constant k_d. This suggests two interaction populations: for example, one where aEpCAM binds with one arm and one where targets are close enough for bivalent binding.

References

1. Protocol: Attaching suspension cells for LigandTracer measurements
2. Bonda S, et al. Front Immunol. 2017. 8(455).
3. Protocol: A typical LigandTracer measurement with MultiDish 2x2