

Data Sheet

SPR Kinetic Affinity Assay

Testing Information

Study Requirements

- Study design, comprising the target receptor (usually a protein) and a reference surface (either a similar but non-binding control protein or an activated-capped surface). Up to 2 additional proteins can be included for testing selectivity, (up to 4 proteins/channels in total).
- Proposed immobilization method, e.g. direct covalent coupling, streptavidin capture of biotinylated proteins, antibody capture of a His-tag, FLAG-tag, Fc-tag or GST-tag protein, or specific antibody capture of a native protein.
- Receptor(s) and control to be immobilized/captured onto the sensor surface; either as purified protein for direct immobilization or possibly as an unpurified tagged-protein preparation to be captured, dependent on study design.
- Minimum of 50 µg of lyophilized receptor and control proteins or 50 µl of 1 mg/ml solution of each in non-TRIS buffered saline (e.g. PBS). A larger quantity / volume of protein (up to 100 µg) may be required for some capture-based study designs.
- *For protein analytes:* a minimum of 100 µg as a lyophilized powder or 100 µl of solution at 1 mg/ml for each analyte to be tested.
- *For small molecule ligands:* a minimum of 0.4 mg of powder or 0.1 ml of 10 mM solution in DMSO for each analyte to be tested.
- It is advisable to include a reference positive control (protein or small molecule) in the test analyte set to validate the assay.
- Molecular weight for the receptor protein and the binding analyte(s) – to predict theoretical analyte binding response.
- Information on solubility and stability for each analyte (if available). Standard solubilization is a 10 mM stock solution in DMSO for small molecules or a 1 mg/ml solution in buffer for proteins, followed by dilution with assay buffer.
- *Note:* Gifford Bioscience can obtain the required receptor, analyte and control proteins, and/or reference compounds if required, for shipment direct to our facility in Birmingham, UK.

Standard Study Processes

- *Receptor immobilization:* optimisation of buffer pH and capture level conditions.
- *Receptor regeneration:* optimisation of serial ligand binding and elution conditions.
- *Ligand affinity measurement:* usually as 5 concentrations of the test analyte over a suitable concentration range (e.g. 1 nM to 1 µM as semi-log or 2-fold dilutions), in duplicate.

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Deliverables

- Graphical plot of analyte binding responses and fitted curves.
- Calculated kinetic affinity constants (k_a , k_d , K_D) for each analyte.
- Calculated equilibrium binding constant (K_D) for each analyte where kinetic affinity parameters k_a and k_d are outside instrument detection parameters.
- Excel summary of fitted binding parameters.
- Report containing detailed methodology, plots of analyte binding responses and summary of fitted binding parameters.
- Electronic lab-book and all accompanying data files.

Turnaround Times

Turnaround times are between two to three weeks for each stage of a study, once samples and materials have been received.

Pricing Structure

For well-validated assay designs, e.g. antibody – antigen interactions, a cost-per-sample basis is applied, based on the number of antibodies(s), antigens and concentrations per assay, with a discounted cost per sample for larger studies.

For novel assay designs not previously validated, an initial *Feasibility Study* is usually necessary to determine if a binding response can be detected, that it is robust and reproducible enough for testing a set of analytes, and that it can be validated with a positive control reference compound.

Once validated, a *Screening Study* (using a single concentration of up to 80 analytes) or a *Kinetic Affinity Study* (using a concentration series for up to 6 analytes) is then run to determine the binding parameters for the set of analytes.

For study costs, download our Price Guide here: [Price Guide](#); [Data Sheets](#); [FAQ's](#) | [Gifford Bioscience](#)