The RAPid 4 Advantage: Real-Time Detection of Antibody Binding by Acoustic Resonance

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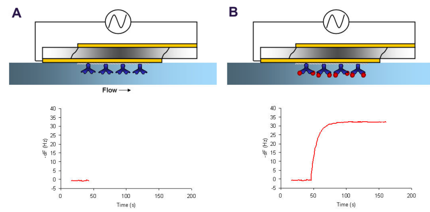
Abstract

Biopharmaceuticals such as monoclonal antibodies have been shown to be effective therapies for complex diseases including cancer. Their development is complicated and heavily reliant on characterising the binding kinetics and affinities of candidate antibodies.
The RAPid 4 acoustic biosensor (TTP LabTech) enables label-free detection of antibody binding in real-time by applying Resonant Acoustic Profiling (RAP). An antigen can be covalently linked to the surface of a quartz sensor. Binding of the antibody to the immobilised antigen causes a change in the oscillation frequency, or acoustic profile of the sensor. This change in frequency can be monitored in real-time, resulting in a fast, accurate measurement of binding kinetics and affinity. In addition, the initial rate of binding can be used to determine unknown protein concentrations.

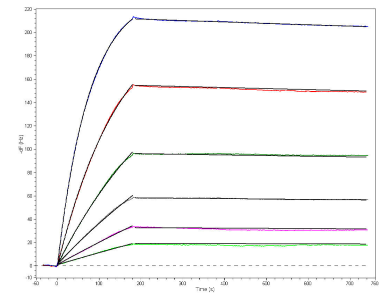
Although optical-based systems dominate the biosensor market, the RAPid 4 device represents a similar but significantly less expensive alternative.
RAP measures only physical binding events and is insensitive to refractive index and colour changes. This enables direct measurement in undiluted crude and complex samples. This advantage simplifies experimental design, and eliminates expensive time-consuming purification of often limited material while delivering high content information.
Efficiency is further refined through automation, typically processing an average of 350 samples per day.

1 Principle of RAP Biosensor Technology



(A) A quartz resonator coated with target receptor is integrated with a liquid system for sample delivery. The target is attached to the sensor surface through direct linkage or capture. With buffer passing over the sensor surface the resonant frequency is measured in real time and displayed on a sensorgram. (B) Samples containing potential binding partners are then applied to the sensor surface. Binding of the ligand to the receptor results in a change in the resonance profile of the resonator. The change in frequency (-dF, Hz) is recorded on the sensorgram to characterize the binding of molecules onto the surface, providing information about the specificity, affinity, kinetics and concentration of molecular binding interactions in real-time. 4 channels can be used in parallel which allows in-line referencing and highest flexibility in assay design by processing up to 4 samples or combinations of samples and control materials.

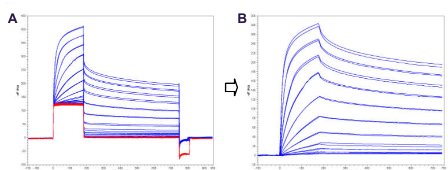
2 Binding Kinetics and Affinities



Media	k_a ($M^{-1}s^{-1}$)	k_d (s^{-1})	K_D (nM)
HBS Buffer	$5.2x10^5$	$5.8x10^{-5}$	0.11

Mouse IgG was injected at several concentrations over a surface coated with a monoclonal rabbit anti-mouse antibody. Using RAPid Workbench analysis software the binding constants were calculated. The RAPid 4 contains a fully automated sample delivery system and is capable of running up to 200 samples per day for kinetic analysis and 3 days of unattended operation.

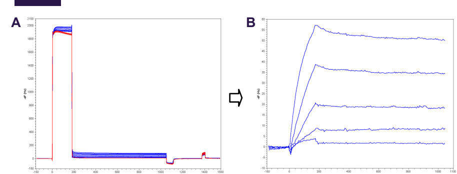
3 Kinetic Characterization in Media



Media	k_a ($M^{-1}s^{-1}$)	k_d (s^{-1})	K_D (nM)
HBS Buffer	$1.9 x10^5$	$4.9 x10^{-4}$	2.5
LB Media	$2.9 x10^5$	$4.5 x10^{-4}$	1.5

Data was collected for antibody binding to a Protein A surface. A human IgG was diluted in 100% LB media and directly injected. A bulk shift was observed due to a change in viscosity (A). However, the RAPid 4 biosensor is not sensitive to refractive index changes. This allows the direct detection of binding in crude or non-purified samples without further sensor calibration and eliminates expensive time-consuming purification of often limited material while delivering high content information. The data are double reference subtracted (B) before applying a 1:1 Langmuir binding model.

4 Binding Data in Periplasmic Extract

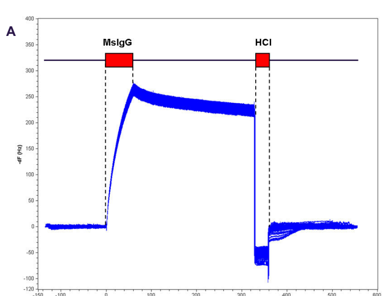


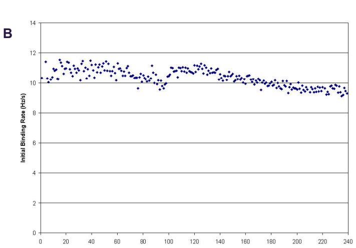
Media	k_a ($M^{-1}s^{-1}$)	k_d (s^{-1})	K_D (nM)
HBS Buffer	$1.01 x10^6$	$8.62 x10^{-4}$	0.85
17% Sucrose	$1.63 x10^6$	$8.47 x10^{-4}$	0.52
Periplasmic Extract	$2.17 x10^6$	$8.92 x10^{-4}$	0.45

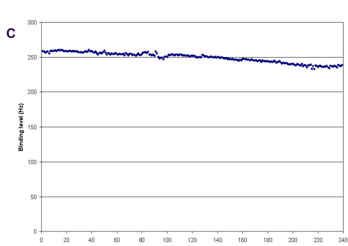
The binding of a single chain variable fragment (scFV) was measured. The raw data (A) and the double reference subtracted data (B) are shown for the protein preparation in HBS buffer + 17% sucrose. The protein-protein interaction for a purified sample in HBS buffer and a crude periplasmic extract were also analysed (data not shown). The kinetic values (Langmuir 1:1) for all three antibody preparations showed good consistency across all conditions.

5 Protein A for antibody capture assays and concentration determination of immunoglobulin

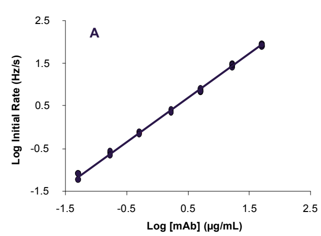
Protein A was tested as an inexpensive alternative to an antibody capture assay. (A) The plot shows 240 consecutive injections of one concentration of a mouse IgG to test the stability of the surface. The surface was regenerated in between using a 30s injection of 5mM HCl. Initial binding rate and binding response level (20 s of the dissociation) were determined as quality criteria of the reproducibility of the assay. (B) The initial binding rate was plotted vs. cycle number. The average was 10.3±0.6 (%RSD 5.8). (C) The binding level was plotted vs. cycle number. The binding average 249.7±7.3 (%RSD 2.9).

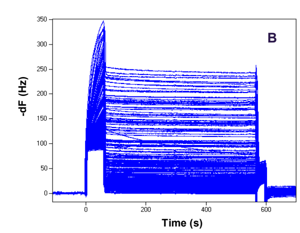


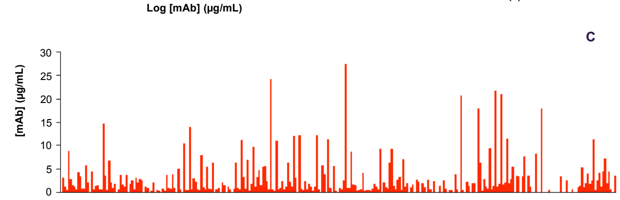




6 Determining Antibody Concentrations







The initial binding rate of an antibody to immobilised antigen is directly proportional to the concentration of active antibody in the sample. First, injections of a known standard at different concentrations were performed and the initial rates determined for each concentration. The resulting calibration curve (A) was used to determine the concentrations of unknown samples (B). The samples were ranked on the basis of concentration or activity (C). The typical throughput of the RAPid 4 instrument for concentration determinations is 350 samples in 24 hours.