Human Fab Capture Kit

BIACORE LABEL-FREE INTERACTION ANALYSIS

Protein biotherapeutics is a growing business and the number of antibody screens performed is increasing rapidly. At the same time, there is an increasing need to make the screening process more rational and cost-effective. Biacore™ systems can simplify and streamline screening by providing essential information and ranking of potential drug leads early in the development process.

Human Fab Capture Kit (Fig 1) is designed for screening and characterization of human Fab antibody fragments using Biacore systems.

Human Fab Capture Kit delivers:

- Rapid and reliable selection of promising Fab fragments
  - High-resolution off-rate ranking: provides essential information early in the process
  - Broad framework specificity: captures kappa and lambda types of human Fab fragments
  - Excellent assay performance: Fab binder with high capture efficiency and stability
- Resource, cost, and time savings
  - No need to identify reagents, and minimum assay development and verification required: preoptimized assay with required solutions provided
  - Minimum need for adjustments between projects: antigen-independent immobilization and regeneration condition

Fig 1. Human Fab Capture Kit is designed for screening and characterization of human Fab antibody fragments using Biacore systems.

Description

Human Fab Capture Kit is intended for use in screening and characterization of human Fab samples, commonly generated by phage display. Samples may include crude cell lysates, periplasmic extracts, or purified Fab fragments. The kit contains Human Fab Binder, immobilization buffer, and regeneration solution. Additional required materials, available from Cytiva, are Sensor Chip CM5, Amine Coupling Kit, and running buffer.

Human Fab Binder, a mixture of monoclonal antibodies recognizing Fab fragment light chains of kappa and lambda types, is first immobilized on a sensor chip surface using immobilization buffer and Amine Coupling Kit. Fab fragments are injected over the surface and captured on the immobilized Human Fab Binder, where their interaction with antigen is studied. Finally, using the regeneration solution included in the kit, the captured Fabs and any associated molecules are removed from the chip surface.

Kit characteristics

Human Fab Capture Kit contains Human Fab Binder and immobilization buffer for 10 immobilizations and regeneration solution for 1000 regeneration injections in Biacore T100 and Biacore A100.

Broad specificity

Human Fab Capture Kit is designed for screening of crude Fab samples in the range of 0.5 to 10 µg/ml. Human Fab Binder binds specifically to the Fab region of human antibodies, and no binding to the Fc region has been observed. It recognizes most subtypes of both kappa and lambda light chains (Table 1) and it does not cross-react with Fab from other species. In addition, nonspecific binding from cell lysate or cell culture medium has not been observed.

Table 1. Binding of light chain subtypes to Human Fab Binder

Framework	Binding verified
Kappa 1	Yes
Kappa 2	Yes
Kappa 3	Yes
Kappa 4	Not available for testing
Lambda 1	Yes
Lambda 2	Not available for testing
Lambda 3	Yes

High surface stability

Immobilized Human Fab Binder captures Fab fragments of both kappa and lambda light chain types with high reproducibility over repeated injections, ensuring reliable results throughout long assay runs (Fig 2 and Table 2).

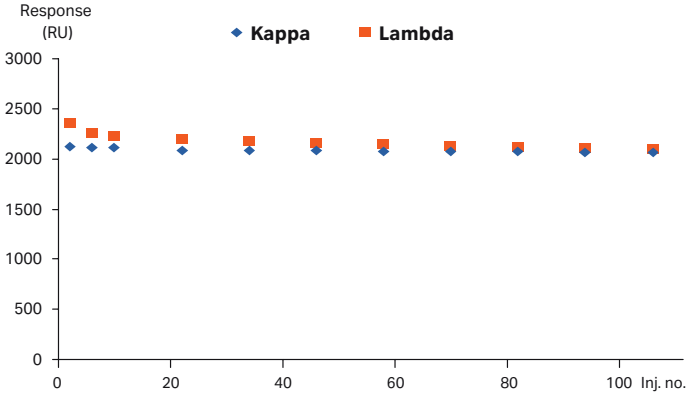

Fig 2. Performance of Human Fab Binder capture during 100 repeated injections.

Table 2. Stability and reproducibility of Human Fab Binder capture

	Fab kappa	Fab lambda
CV (response level)	1%	4%
Activity after 100 cycles	97%	89%

Applications

Screening

Faster selection of stable binders from a set of Fab fragments can be achieved by ranking based on off-rates for the Fab-antigen interaction. Off-rate ranking provides good estimates of the actual dissociation constants and gives kinetic information already in the screening phase. Using Human Fab Capture Kit, off-rate ranking of human Fabs is performed by capturing Fab samples on the immobilized Human Fab Binder, injecting the antigen, and measuring the interaction. In order to ensure high ranking resolution, a blank cycle, using buffer instead of antigen, is run with every Fab to allow for blank subtraction.

This gives a throughput of:

- 384 Fab samples (768 cycles) in 24 h using Biacore A100
- 48 Fab samples (96 cycles) in 24 h using Biacore T100

For maximum throughput, report-point based screening may be chosen, where a binding level is measured instead of off-rate determination. This doubles the throughput as the blank cycles can be omitted.

The following data demonstrate that Human Fab Capture Kit is suitable for screening of crude Fab samples.

Table 3 and Figure 3 show an off-rate ranking experiment, performed on Biacore A100, with Fabs diluted to 0.5 µg/ml in cell culture medium interacting with an antigen (5 µg/ml) of relative molecular mass (M_r) 15 000 (i.e., 15 kDa). After reference and blank subtractions, the sensorgram data were fitted to a 1:1 binding model. The samples were ranked from slow to fast off-rates.

Table 3. Ranking of Fab samples in cell culture medium based on off-rates (k_d) for the Fab-antigen interaction

Ranking position	Sample	k_d (s^{-1})	Framework
1	Fab A	2.7×10^{-4}	Lambda 3
2	Fab B	8.5×10^{-4}	Lambda 3
3	Fab C	1.8×10^{-3}	Lambda 3
4	Fab D	7.2×10^{-3}	Kappa 1
5	Fab E	4.0×10^{-2}	Kappa 1

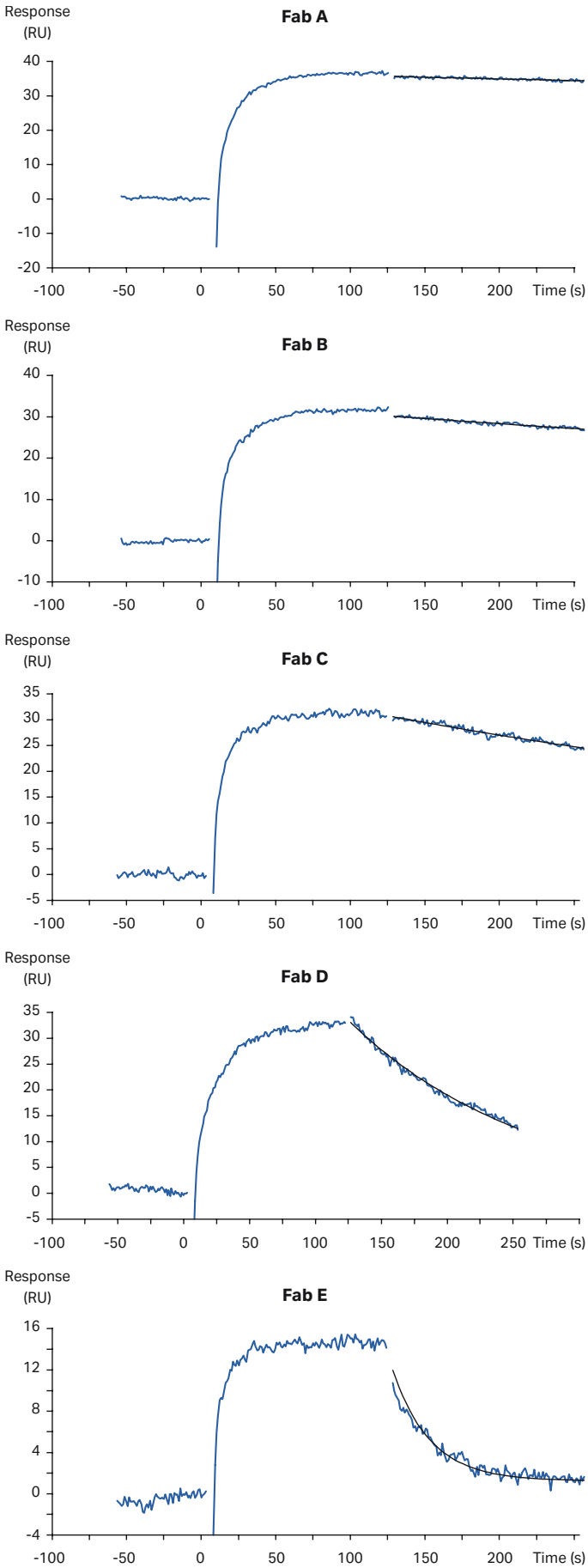

Fig 3. Sensorgrams from a screening on Biacore A100 of Fab samples in cell culture medium.

Kinetic characterization

For kinetic characterization, Fab fragments are first captured on immobilized Human Fab Binder followed by injection of analyte at a series of concentrations. Most types of analytes are suitable, and the kit has been verified for proteins and peptides from M_r 10 000. The required sample volume is typically 100 µl.

The following data show that Human Fab Capture Kit can be used to characterize a variety of different Fab-antigen interactions with a broad range of kinetic characteristics.

The interactions of three different Fab fragments with antigen (M_r 15 000) were characterized on Biacore T100 (Fig 4; Table 4). The Fab fragments were diluted to 1 µg/ml, and an antigen concentration of 0.025 to 0.4 µM was used. Sensorgram data were fitted to a 1:1 binding model.

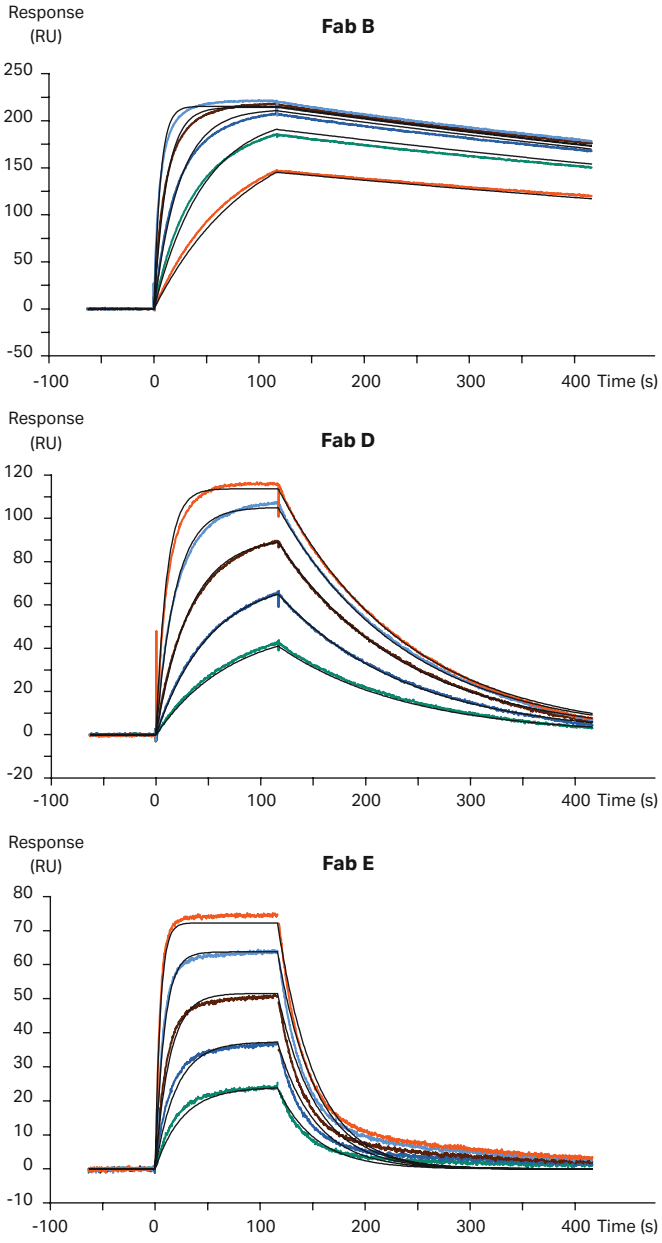

Fig 4. Sensorgrams from kinetic characterization of Fab fragments on Biacore T100.

Table 4. Results from kinetic characterization of Fab samples on Biacore T100

Sample	k_a ($M^{-1}s^{-1}$)	k_d (s^{-1})	K_D (M)
Fab B	4.1×10^5	7.2×10^{-4}	1.8×10^{-9}
Fab D	2.3×10^5	8.2×10^{-3}	3.6×10^{-8}
Fab E	4.4×10^5	2.7×10^{-2}	6.2×10^{-8}

Figure 4 and Table 4 show a full kinetic characterization run using the classical multicycle approach. Single-cycle kinetics and 2-over-2 kinetics are two alternative approaches that improve throughput and save reagents, while maintaining data quality. Human Fab Capture Kit can be used independently of the approach chosen, however, the support for the different methods differ somewhat between Biacore systems.

Acknowledgements

Fab samples and antigen for the application examples were kindly provided by MorphoSys AG, Munich, Germany.

Ordering information

Product	Quantity	Code no.
Human Fab Capture Kit[1]	1	28-9583-25

[1] Includes Human Fab Binder and immobilization buffer for 10 immobilizations and regeneration solution for 1000 regeneration injections.

cytiva.com/biacore

CY13669-10Aug20-DF