Biacore™ cap-tag capture kit

LABEL-FREE INTERACTION ANALYSIS

Biacore™ cap-tag capture kit provides a streamlined workflow that enables kinetic and affinity analysis for a wide variety of protein interactions with minimal assay development using a fully optimized capture and regeneration protocol (Fig 1, Fig 2). With this kit, you attach your protein of interest to Series S Sensor Chip CAP by first conjugating Biacore cap-tag (a short oligonucleotide tag) to your protein, which then binds to the complementary oligo on the sensor chip. Then, reversibly capture your Biacore cap-tag protein for protein interaction analysis in Biacore systems. The regeneration solution provided in Biacore regeneration kit CAP removes your protein and any bound analyte from the surface after each analysis cycle. No assay development is necessary to find suitable immobilization or regeneration conditions. The sensor chip is then ready to capture the same or different proteins at least 150 times—making it a cost-effective choice.

Biacore cap-tag capture kit offers the following benefits:

- Simple protein capture regardless of tag.
- Savings in time and effort. No need to develop attachment or regeneration conditions.
- Reusable and cost-effective.
- Easy to follow instructions and predefined Biacore Insight software methods.

Description

Biacore cap-tag capture kit series s is a combination of three different products containing all the reagents required for conjugation and capture of Biacore cap-tag ligands:

- Biacore cap-tag conjugation kit, containing Biacore conjugation buffer, Biacore ligand activator and Biacore cap-tag.
- Amersham™ MicroSpin™ G-50 columns for buffer exchange and purification.
- Series S Sensor Chip CAP.

Biacore cap-tag capture kit series s should be used alongside:

- Biacore regeneration kit CAP, containing stocks for preparing regeneration solution for Series S Sensor Chip CAP.

Fig 1. Biacore cap-tag capture kit series s and Biacore regeneration kit CAP for protein interaction analysis on Biacore 8 series, Biacore 1 series, Biacore T200 and Biacore S200 SPR systems.

The products are also available for standalone purchase to provide flexibility for your assay set up. If you already have solutions or chips available in the lab there's no need to purchase the entire kit.

Detailed instructions and predefined software methods for Biacore Insight kinetics analysis and evaluation are provided for an easy to follow workflow from protein to evaluated kinetic data.

Biacore cap-tag capture kit was developed for protein interaction studies on Biacore 8 series, Biacore 1 series, Biacore T200, and Biacore S200 SPR systems. Potential applications for the kit include yes/no binding, affinity, kinetics and more.

SPR optimized. Tag. Capture. Reuse.

Kinetic characterization with full support

Figure 2 shows the workflow for using Biacore cap-tag capture kit and Biacore regeneration kit CAP. An example application is kinetic characterization of protein-protein interactions. Here we describe the use of the kit to characterize binding of a nanobody to the receptor binding domain (RBD) of the SARS-CoV-2 spike proteins.

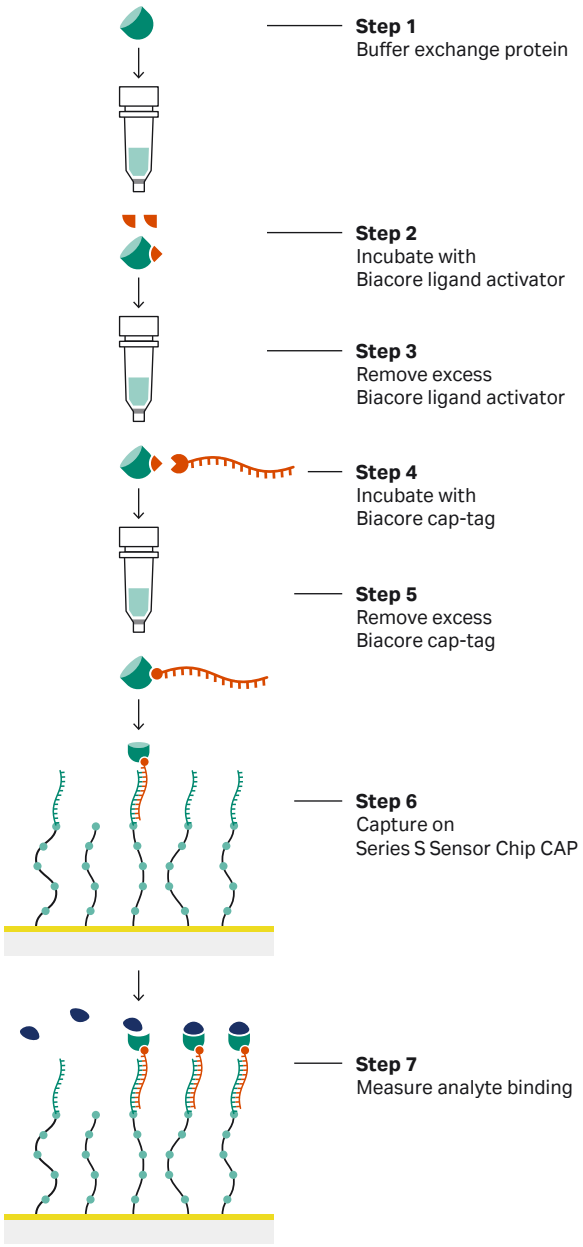

Fig 2. Biacore cap-tag capture protocol overview.

First, the RBD ligand conjugate was prepared following the steps outlined in Figure 2. Series S Sensor Chip CAP was rehydrated in parallel with the conjugation. Next, capture level scouting was performed using the predefined Biacore Insight software method (Fig 3A). A dilution factor of 3000 provided a suitable capture level (~ 20 RU capture) to achieve a theoretical maximum analyte binding capacity of 10 RU (Fig 3B). Then, a kinetics analysis was completed using the predefined Biacore Insight software method for Biacore Single-Cycle Kinetics (SCK)™ analysis. This provided a first estimation of the kinetics constants (Fig 3C). By lowering the concentration range and extending the dissociation time in a second run, a more exact determination of the kinetics was obtained (Fig 3D).

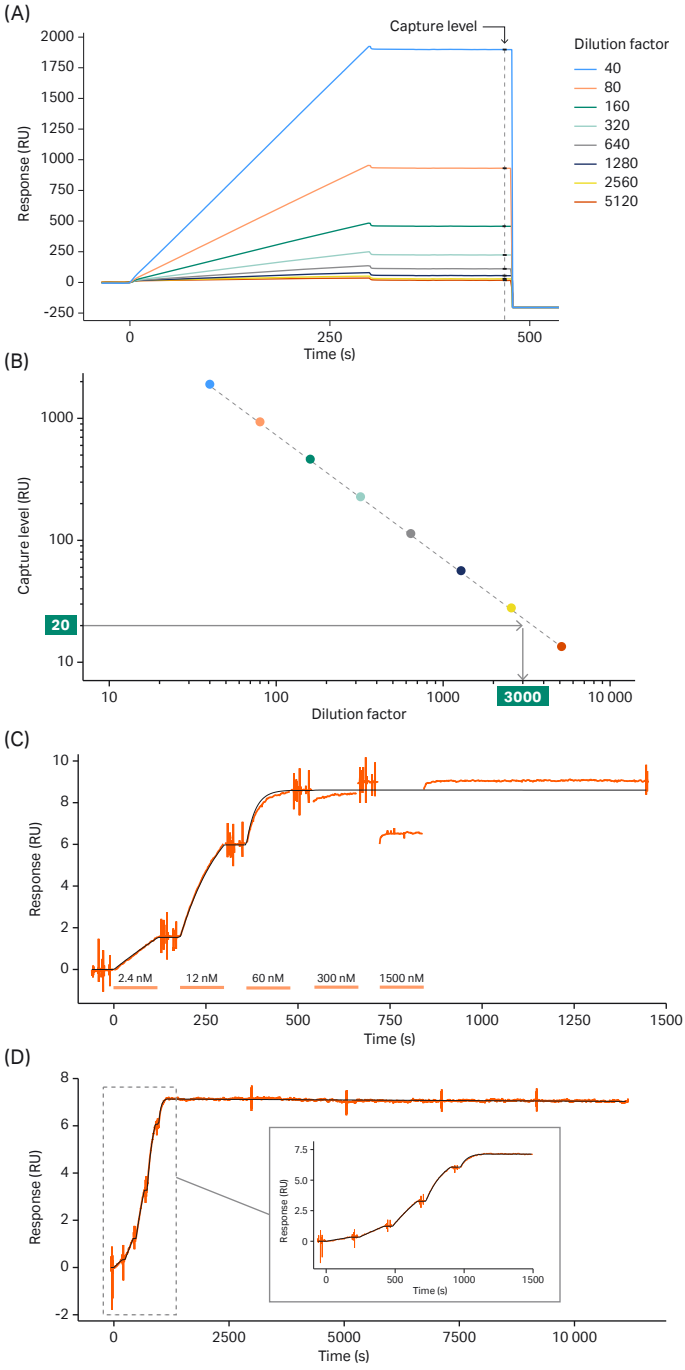

Fig 3. Characterization of a nanobody binding to RBD using Biacore cap-tag capture kit. (A) RBD ligand conjugate capture level scouting sensorgram. (B) Log-log plot showing capture level vs dilution factor for the RBD ligand conjugate. (C) Initial SCK run using the predefined Biacore Insight software method. (D) Refined SCK run using a lower analyte concentration range and longer dissociation time. SCK sensorgrams in orange with 1:1 binding model fit in black.

Capture and characterize multiple types of proteins—regardless of tag

Biacore cap-tag capture kit is designed for protein-protein interaction analysis using ligand proteins with molecular weight 30–160 kDa. No pre-existing tags are needed—the kit chemistry targets primary amines (for example lysine side chains) and thiols on the ligand protein.

Series S Sensor Chip CAP and Biacore cap-tag contain deoxyribo-oligonucleotides. Therefore, the kit is not suitable for work with DNA-binding proteins or enzymes that degrade DNA.

The kinetics of target-specific nanobodies binding to three different ligand conjugates prepared and analyzed using Biacore cap-tag capture kit on Biacore 1K+: green fluorescent protein (GFP), glutathione s-transferase (GST), and maltose binding protein (MBP) are shown in Figure 4. The obtained kinetics constants (k_a and k_d) agree with what we have seen using other capture methodologies (Table 1).

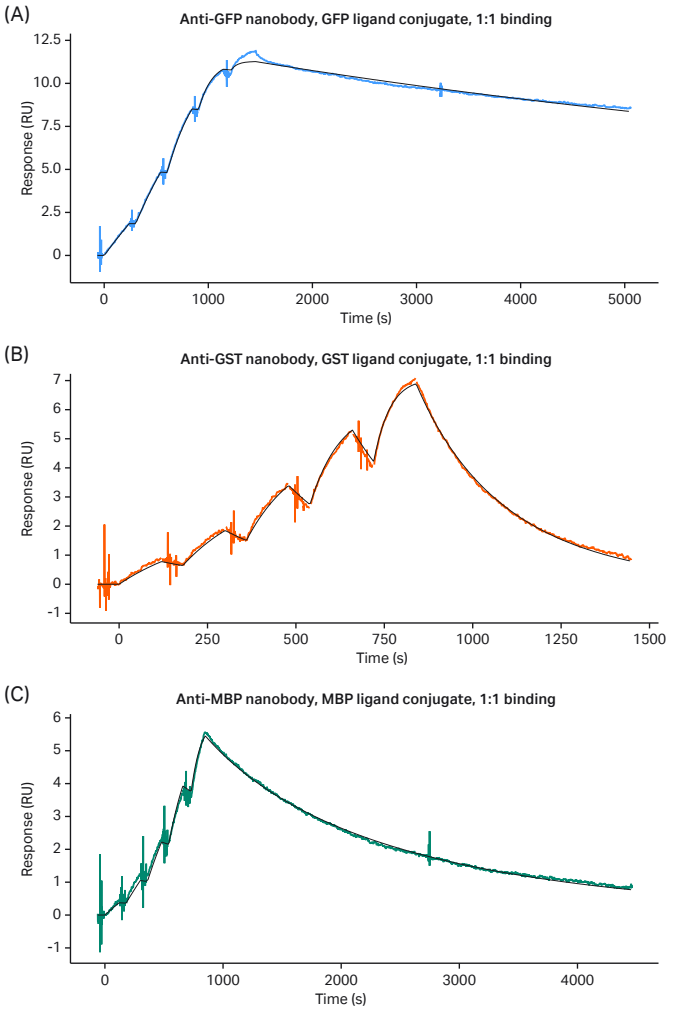

Fig 4. Biacore 1K+ SCK analysis of target-specific nanobodies binding to ligand conjugates. Reference- and blank-subtracted sensorgrams in color, with 1:1 binding model fit in black. Ligand conjugates were prepared using Biacore cap-tag conjugation kit and captured on Series S Sensor Chip CAP, before a concentration series of target-specific nanobody was injected. (A) GFP ligand conjugate and anti-GFP nanobody. (B) GST ligand conjugate and anti-GST nanobody. (C) MBP ligand conjugate and anti-MBP nanobody.

Table 1. Kinetics constants (n=1) from 1:1 binding model fits shown in Figure 4, compared to example data obtained using an alternative capture methodology (Biotin CAPture Kit)

	Biacore cap-tag capture		**Biotin CAPture Kit**	
	k_a (1/Ms)	k_d (1/s)	k_a (1/Ms)	k_d (1/s)
GFP and anti-GFP nanobody	1.1×10^7	8.8×10^{-5}	8.8×10^6	1.0×10^{-4}
GST and anti-GST nanobody	4.2×10^5	4.5×10^{-3}	3.7×10^5	3.0×10^{-3}
MBP and anti-MBP nanobody	3.2×10^5	8.3×10^{-4}	4.1×10^5	6.8×10^{-4}

Streamline assay development—fast results, lower cost

Developing an assay using amine coupling can be an iterative process. Steps such as pH scouting to find a suitable coupling solution for effective ligand preconcentration and regeneration scouting to find a solution that removes the analyte without damaging the ligand may need to be repeated multiple times before satisfactory conditions are found. This increases both the time spent on the instrument and the cost of materials, as multiple sensor chips may need to be used and discarded. Additionally, some ligands are unstable and may not tolerate the low pH used during amine coupling or the repeated exposure to regeneration solutions.

With Biacore cap-tag capture kit, you can speed up your time to results while reducing instrument time, which can mean significant savings when paying for access to Biacore systems in core facilities. Biacore cap-tag conjugation is done away from the instrument at your own bench and takes around 3–4 h. Your protein is then ready for capture on Series S Sensor Chip CAP without any additional assay development or optimization—so it's possible to obtain kinetic information for your interaction in less than a day of instrument time. The kit is also used alongside an optimized regeneration solution that allows reuse of your sensor chip at least 150 times.

Biacore cap-tag capture kit streamlines assay development for research projects with its one simple protocol for a multitude of proteins regardless of existing tag, pH stability, or regeneration requirements (Fig 5). The easy to follow instructions guide even beginner users all the way from start to finish—from protein to evaluated kinetic data. This frees up time and allows for more projects because of efficiently managed instrument time.

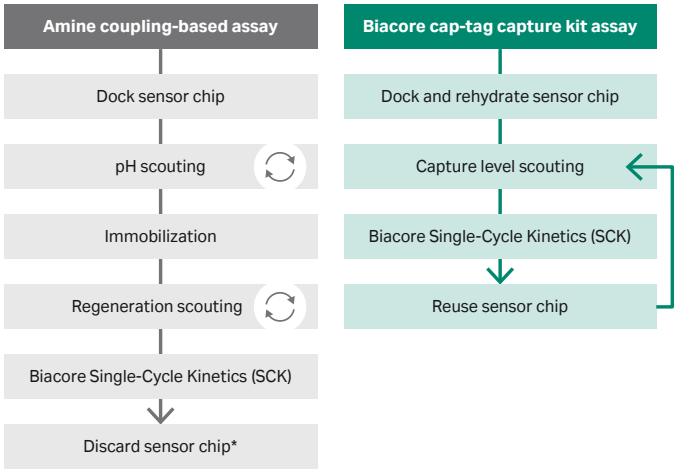

Fig 5. Instrument steps of an amine-coupling-based assay compared to a Biacore cap-tag capture assay. *Multiple cycles can be run on the same surface. However, the flow cell cannot be reused with a different ligand.

Advance multiple projects with one capture approach

Biacore cap-tag capture kit allows for analysis of multiple proteins using the same kit. Normally, the yield of ligand conjugate is high, meaning that it must be diluted many times to get capture levels suitable for kinetic analysis. For example, if a 50 µL ligand conjugate is diluted 3000 times it's possible to prepare sufficient diluted ligand conjugate solution to run over 3400 cycles on a Biacore 1 series instrument.

Series S Sensor Chip CAP can be reused at least 150 times per flow cell using Biacore regeneration kit CAP. The capture level and analyte binding level for three ligand conjugates and their respective nanobody binders over 150 cycles are shown in Figure 6. The surface is incredibly stable for both the GFP and MBP ligand capture and nanobody binding, with a small downwards trend for the GST capture and nanobody binding, which is likely related to the stability of the protein itself.

One Biacore regeneration kit CAP contains sufficient solutions to prepare a total of 20 mL regeneration solution. The volume required per injection will vary depending on Biacore system, vial or plate type, and whether pooling is used, but is typically 40–50 µL per injection. This means ~ 400–500 regeneration injections can be performed using one kit.

Get more from your sensor chip

Biacore cap-tag capture kit is designed with flexibility in mind. It's possible to run multiple projects for many different protein interactions with one kit. We understand that no project is the same and that the exact number of uses will vary depending on how many flow cells or channels are used, how often regeneration is needed, capture levels, number of different protein ligands and how samples are pooled in the instrument. In Table 2 we provide an estimation of how many interactions can be studied per Series S Sensor Chip CAP.

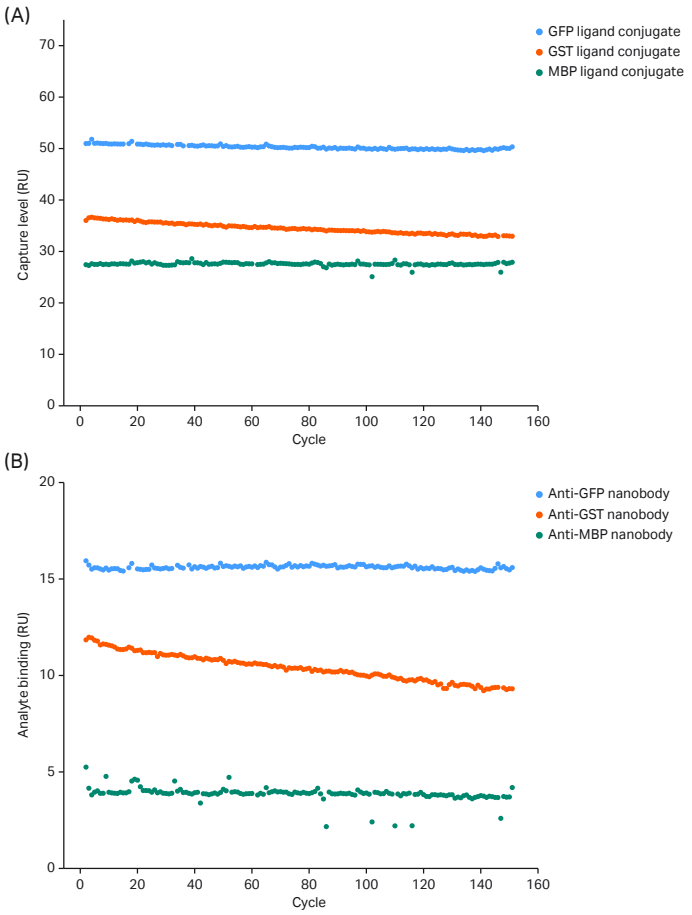

Fig 6. Ligand conjugate capture level (A) and analyte binding response (B) for three different ligand conjugates and their target-specific nanobodies.

Table 2. Number of interactions per Series S Sensor Chip CAP used in combination with Biacore cap-tag conjugation kit and Biacore regeneration kit CAP.

Assay set up	Number of interactions per chip	
	Biacore 1 series*	Biacore 8 series
Yes/no binding	400	400 (1000†)
Single cycle kinetics	200	200 (530†)

*Utilizing all six flow cells with three different ligands per cycle.

†To get the most out of one Series S Sensor Chip CAP on Biacore 8 series, we recommend purchase of more than one Biacore regeneration kit CAP.

Get the most out of your instrument time

Biacore cap-tag capture kit allows for more efficient use of instrument time. Once you have the conjugate and the rehydrated sensor chip you are ready to go— there's no need to spend precious instrument time on pH scouting and regeneration scouting. Just open our predefined software methods, dilute your samples and start the run. Performing a capture level scouting takes around 2 h of unattended run time, or around 30 min using ***Interactive run***. An SCK run typically takes around 2–2.5 h for one interaction and 3.5 h if different ligands are captured on different flow cells. You can easily obtain kinetics information within 5 h for up to three different ligands using Biacore 1 series, or eight different ligands using Biacore 8 series.

Ordering information

Product	Product code
Biacore cap-tag capture kit series s Includes: Biacore cap-tag conjugation kit Series S Sensor Chip CAP Amersham MicroSpin G-50 Columns (50 pack)	29873737
Biacore regeneration kit CAP	29805821

Related products	Product code
Biacore cap-tag conjugation kit Includes: Biacore cap-tag Biacore ligand activator Biacore conjugation buffer 17 mL	29805822
Series S Sensor Chip CAP	29805820
Amersham MicroSpin G-50 Columns (50 pack)	27533001

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