

His Capture Kit

His Capture Kit is designed for capture of histidine-tagged proteins by an anti-histidine antibody and provides an alternative to capture on nickel-chelated nitrilotriacetic (NTA) groups. Capture of histidine-tagged recombinant proteins is an attractive approach for protein attachment in Biacore systems since physiological conditions can be used during the whole procedure. In addition, capture of tags generates a directed structural orientation of the protein on the surface, potentially offering optimal site exposure.

Depending on the nature of the interacting molecules, the approach used with His Capture Kit can give lower nonspecific binding and/or higher binding stability. The anti-histidine antibody included in the kit is a monoclonal antibody (MAb) directed against polyhistidine tags on the C or N terminus of a protein.

His Capture Kit delivers:

- Savings in time and effort: Minimum assay development with no need to identify reagents or verify assay conditions
- Maximum convenience: Optimized protocol and all required solutions for the efficient capture of histidine-tagged proteins included

Attachment by capture has several advantages over covalent immobilization. Tagged proteins can be captured from crude media solutions, requiring little or no sample preparation. Regeneration becomes generic and the need for assay development is minimized. Histidine tags are widely used since they are small and rarely interfere with the function, activity, or structure of the target proteins. Two approaches for capture of histidine-tagged molecules are frequently used: Capture by an anti-histidine antibody; and capture on nickel-chelated NTA groups. The choice between these alternatives often depends on the nature of the interacting molecules to be studied. GE Healthcare offers products for both approaches, His Capture Kit and Sensor Chip NTA with NTA Reagent Kit (see Data file 29-0079-27).



Fig 1. His Capture Kit allows convenient capture of histidine-tagged proteins on Biacore sensor chips.

Description

His Capture Kit contains anti-histidine antibody, immobilization buffer, and regeneration solution. Additional materials needed, available from GE Healthcare, are a carboxyl-derivatized sensor chip (Sensor Chip CM5, CM4, CM3, or C1), Amine Coupling Kit, and running buffer.

The antibody recognizes polyhistidine tags localized at the C or N terminus of a protein.

For interaction analyses, the anti-histidine antibody is first covalently immobilized onto the sensor chip surface using the included immobilization buffer and Amine Coupling Kit. The histidine-tagged ligand is then injected and captured by the immobilized anti-histidine antibody, after which the analyte sample is injected. By using the optimized regeneration solution included in the kit, the captured histidine-tagged ligand and any associated molecules are removed from the sensor chip surface making it ready for a new experimental cycle. His Capture Kit can be used in any Biacore system (except Biacore Flexchip) and is sufficient for 10 immobilizations and 1000 regeneration injections in Biacore T200, Biacore T100, Biacore 4000, and Biacore A100.



Capture stability

There are several ways to increase the capture stability for histidine-tagged proteins. An efficient way is to lower the amount of captured tagged protein (Fig 2). This increases capture stability since the lower number of occupied binding sites on the surface increases the probability that dissociating ligands will rebind to a neighboring antibody. Lower capture levels can be obtained by lowering the protein concentration or by shortening the contact time.

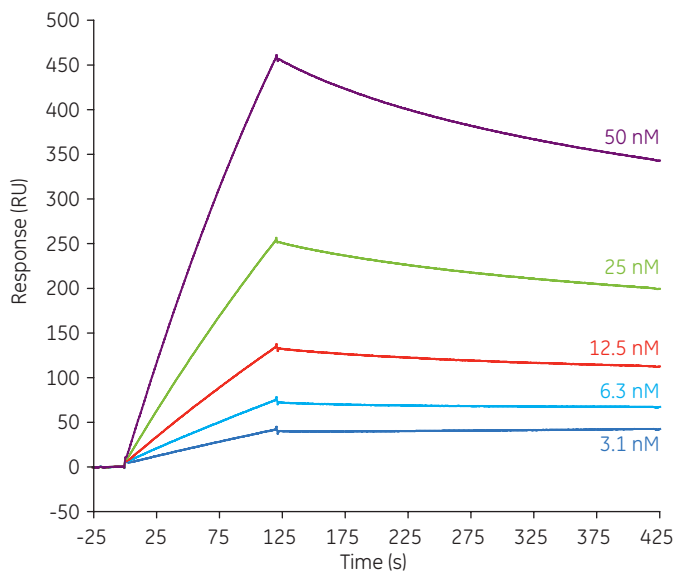


Fig 2. Injections of different concentrations of histidine-tagged PARP10 (0.8 to 6.4 $\mu\text{g}/\text{ml}$) over an anti-histidine antibody surface prepared with His Capture Kit.

Application

His Capture Kit is widely applicable and the anti-histidine antibody surface can be used for all types of Biacore analyses, from binding studies and screening to concentration determinations and kinetic characterization. His Capture Kit is compatible with different types of histidine-tagged biomolecules and is suitable for a wide range of analyte molecules, from low molecular weight compounds to large proteins.

In the following application example, the kinetics of papain (M_r 23 400) binding to cystatin B (M_r 11 000) was determined using His Capture Kit in a Biacore T100 system. Anti-histidine antibody was immobilized (12 000 RU) in flow cells 1 and 2 on Sensor Chip CM5 and 30 RU of cystatin B (6 nM) was captured on the surface. Papain (0.125 to 2 nM) was then injected over the captured protein (Fig 3). The data was fitted to a 1:1 binding model with high accuracy, as demonstrated by the statistical parameters such as the reported Chi^2 value ($<0.1 \text{ RU}^2$). The anti-histidine capture approach thus enabled a stable capture allowing confident generation of kinetic values.

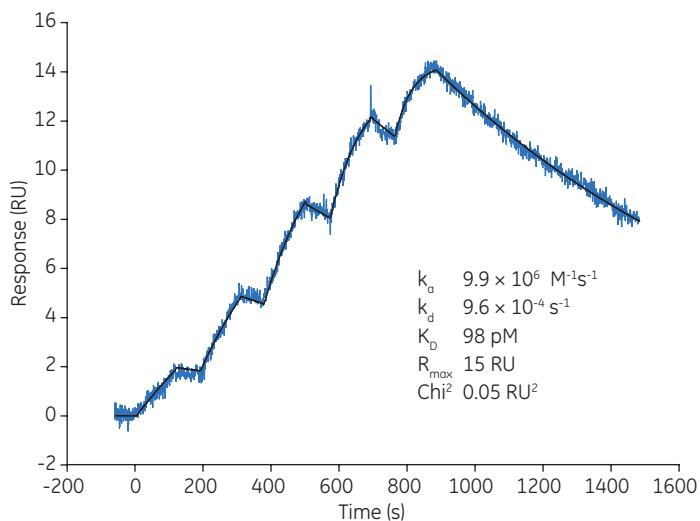


Fig 3. Kinetic characterization of papain (0.125 to 2 nM) binding to cystatin B captured onto an anti-histidine antibody surface prepared with His Capture Kit.

Acknowledgements

PARP10 was kindly provided by Johan Weigelt, Chief Scientist, at SGC, Karolinska Institute, Stockholm, Sweden. Cystatin B and papain were kindly provided by Prof. Ingemar Björk's group at the Swedish University of Agricultural Sciences, Sweden.

Ordering information

Product	Quantity	Code number
His Capture Kit	1	28-9950-56

Related products

Series S Sensor Chip CM5*	3	BR-1005-30
Series S Sensor Chip CM4*	3	BR-1005-34
Series S Sensor Chip CM3*	3	BR-1005-36
Series S Sensor Chip C1*	3	BR-1005-35
Sensor Chip CM5†	3	BR-1000-12
Sensor Chip CM5†	1	BR-1003-99
Sensor Chip CM4†	3	BR-1005-39
Sensor Chip CM3†	3	BR-1005-41
Sensor Chip C1†	3	BR-1005-40
Amine Coupling Kit†	1	BR-1000-50
Amine Coupling Kit, type 2‡	1	BR-1006-33
Series S Sensor Chip NTA*	3	BR-1005-32
Series S Sensor Chip NTA*	1	28-9949-51
Sensor Chip NTA†	3	BR-1000-34
Sensor Chip NTA†	1	BR-1004-07
NTA Reagent Kit	1	28-9950-43

* For use in Biacore 4000, Biacore A100, Biacore T200, Biacore T100. Format compatible with Biacore S51, but no system support available

† For use in all other Biacore systems, except Biacore Flexchip

‡ For use in Biacore 4000, Biacore A100 and Biacore S51

Related literature

Approaches for capture of histidine-tagged proteins in Biacore systems, Application Note	29-0079-29
Sensor Chip NTA, Series S Sensor Chip NTA, NTA Reagent Kit, Data file	29-0079-27
Sensor Surface Handbook	BR-1005-71

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