# Approaches for capture of histidine-tagged proteins in Biacore systems

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# Approaches for capture of histidine-tagged proteins in Biacore™ systems

**Capture of histidine-tagged proteins for interaction analysis in Biacore systems can be achieved either via nickel ($Ni^{2+}$) chelation of nitrilotriacetic acid (NTA) or by covalently coupling an anti-histidine antibody to the sensor surface. Both alternatives give high binding capacities and allow simple regeneration and reuse for multiple cycles. GE Healthcare provides dedicated products supporting both approaches, offering easy set-up and use in Biacore systems.**

**The features of the two approaches are discussed here, and guidelines for working with histidine-tagged proteins in Biacore assays are provided.**

## Introduction

One of the tags most widely used to facilitate purification of recombinant proteins is the histidine tag, which typically consists of six consecutive histidine residues added to the C- or N-terminus of the protein.

Attachment of proteins to sensor surfaces via capture of a histidine tag is attractive since physiological conditions can be used during the capture procedure. Another advantage is that histidine capture generates a uniform structural orientation of the ligand. Two capture approaches are frequently used: Capture on nickel-chelated NTA groups, and capture by immobilized anti-histidine antibodies. In both cases, a generic regeneration method can be applied for all types of proteins.

In this Application note tips and hints for working with histidine-tagged proteins in Biacore systems are described.

## Materials and methods

All experiments were performed using Biacore T100 or Biacore 4000. The following buffers were used: HBS-P+ or PBS-P+, with 50 µM EDTA added, for Sensor Chip NTA, and HBS-EP+ for His Capture Kit.

**Fig 1.** Sensor Chip NTA is used for the study of molecular interactions between histidine-tagged proteins immobilized on the chip and numerous analytes.

### *Capture and analysis using Sensor Chip NTA*

Sensor Chip NTA was activated by injecting a solution of $Ni^{2+}$ ions followed by capture of the histidine-tagged protein. The analyte solution was injected and the binding signal(s) monitored. The NTA surface was then regenerated by a pulse of EDTA. Running conditions for the use of Sensor Chip NTA in this study are listed in Table 1.

**Table 1.** Running conditions for kinetic characterization of analytes binding to their respective ligands integrin α1β9, MBP, and cystatin B captured on Sensor Chip NTA

| Step | Reagent | Concentration | Flow rate (µl/min) | Contact time (min) |
|---|---|---|---|---|
| Conditioning* | EDTA | 350 mM | 30 | 1 |
| Activation | $NiCl_2$ | 0.5 mM | 10 | 1 |
| Extra wash | EDTA | 3 mM | 30 | N/A |
| Capture | Histidine-tagged protein | 0.6 to 20 µg/ml | 10 | 1 |
| Sample analysis | Analyte | Varying | 30 | 2 |
| Regeneration | EDTA | 350 mM | 30 | 1 |

* Only at the beginning of each experiment

### *Capture using anti-histidine antibody*

The anti-histidine antibody provided in His Capture Kit was diluted to 50 µg/ml in the immobilization buffer included in the kit and covalently coupled to Sensor Chip CM5 by standard amine coupling to a level of approximately 12 000 RU. Parameters for immobilization of the antibody and subsequent analysis of the histidine-tagged proteins are listed in Tables 2 and 3.

**Table 2.** Immobilization conditions for anti-histidine antibody

| Step | Reagent | Flow rate (µl/min) | Contact time (min) |
|---|---|---|---|
| Activation | EDC/NHS | 5 | 7 |
| Ligand | Histidine-tagged protein | 5 | 7 |
| Deactivation | Ethanolamine | 5 | 7 |

**Table 3.** Running conditions for kinetic characterization of analytes binding to their respective ligands integrin integrin α1β9, MBP, and cystatin B captured on an anti-histidine antibody surface

| Step | Reagent | Concentration | Flow rate (µl/min) | Contact time (min) |
|---|---|---|---|---|
| Capture | Histidine-tagged protein | 0.6 to 20 µg/ml | 10 | 1 |
| Sample injection | Analyte | Varying | 30 | 2 |
| Regeneration | Glycine pH 1.5 | 10 mM | 30 | 1 |

### *Use of reference surface*

When working with capture to anti-histidine antibodies, the reference surface should be immobilized with anti-histidine antibody using settings identical to those used for the active surface.

When working with Sensor Chip NTA, the reference surface should be a non-activated flow cell/detection spot that has not been subjected to $Ni^{2+}$ ions. For a surface loaded with $Ni^{2+}$ ions, but without ligand, there is a risk of nonspecific binding from complex sample matrices at a level that does not match the active surface.

As the remaining amount of unoccupied nickel-chelated groups on Sensor Chip NTA may increase the probability for analyte binding to the active surface, it is recommended to start work on Sensor Chip NTA with a control experiment checking for unwanted analyte binding on an activated surface.

## Results and discussion

### *Capture stability*

Capture stability varies between histidine-tagged proteins, largely depending on the nature of the protein itself and on the microenvironment of the tag in particular. Other factors that influence the stability of the capture are type of capturing surface and amount of captured protein. In order to increase stability, capture levels should generally be kept as low as possible.

Three histidine-tagged proteins (Integrin α9β1, $M_r$ 200 000; maltose binding protein [MBP], $M_r$ 43 000; cystatin B, $M_r$ 11 000) were captured on both Sensor Chip NTA and an anti-his antibody surface. The sensorgrams are shown in Figure 2. Integrin α9β1 (Fig 2A) and MBP (Fig 2B) do not give stable baselines with either of the capture approaches. Cystatin B, on the other hand, gives a stable baseline with Sensor Chip NTA while capture with anti-histidine antibody is less stable (Fig 2C). These are examples where assay development is necessary to obtain a stable binding of the histidine-tagged protein.

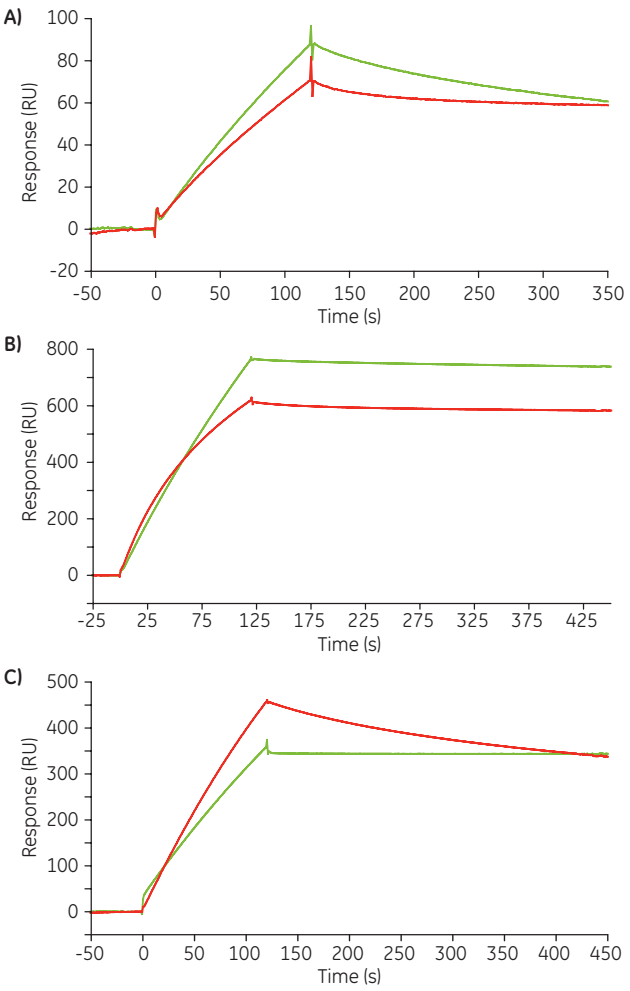


**Fig 2.** Capture of histidine-tagged proteins on Sensor Chip NTA (green curve) and anti-his antibody surface (red curve) in Biacore T100. **A)** Integrin α9β1 1.25 µg/ml; **B)** MBP 20 µg/ml; **C)** cystatin B 0.6 µg/ml.

As mentioned, there are several ways to increase the binding stability of captured histidine-tagged proteins. An efficient and simple approach is to decrease the protein load. This provides a larger number of unoccupied binding sites on the surface which, in turn, increases the probability for rebinding of dissociating proteins.

A lower capture level can be obtained by adjustment of the protein concentration or by a shorter contact time. Examples of injections of varying concentrations of histidine-tagged proteins over Sensor Chip NTA and anti-histidine antibody are shown in Figures 3 and 4.

In all these examples, it is clear that the stability increases with a lower capturing level. In most cases, it is easy to achieve a level with excellent stability.

At 50 nM, cystatin B dissociates fairly rapidly from the surface, 0.35 RU/s (Fig 4B, purple curve). When the concentration is lowered the dissociation rate is reduced, and at 6.3 nM (light blue curve), the dissociation of protein from the surface is less than 0.01 RU/s, which is sufficient for high quality kinetic studies.

For some applications, however, a compromise between a capture level high enough to give a good analyte response and sufficient stability must be made.

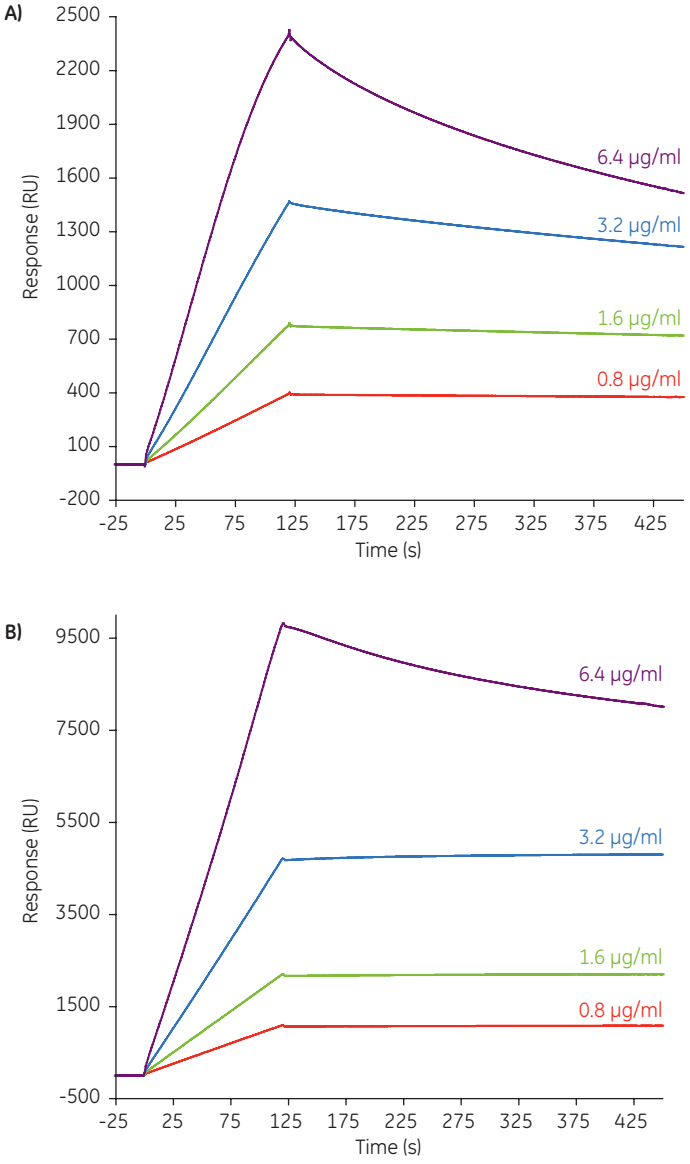


**Fig 3.** Concentration series for capture of histidine-tagged proteins on Sensor Chip NTA. **A)** Green Fluorescent Protein (GFP, $M_r$ 27 000, 0.8 to 6.4 µg/ml); **B)** PARP10 ($M_r$ 25 000, 0.8 to 6.4 µg/ml).

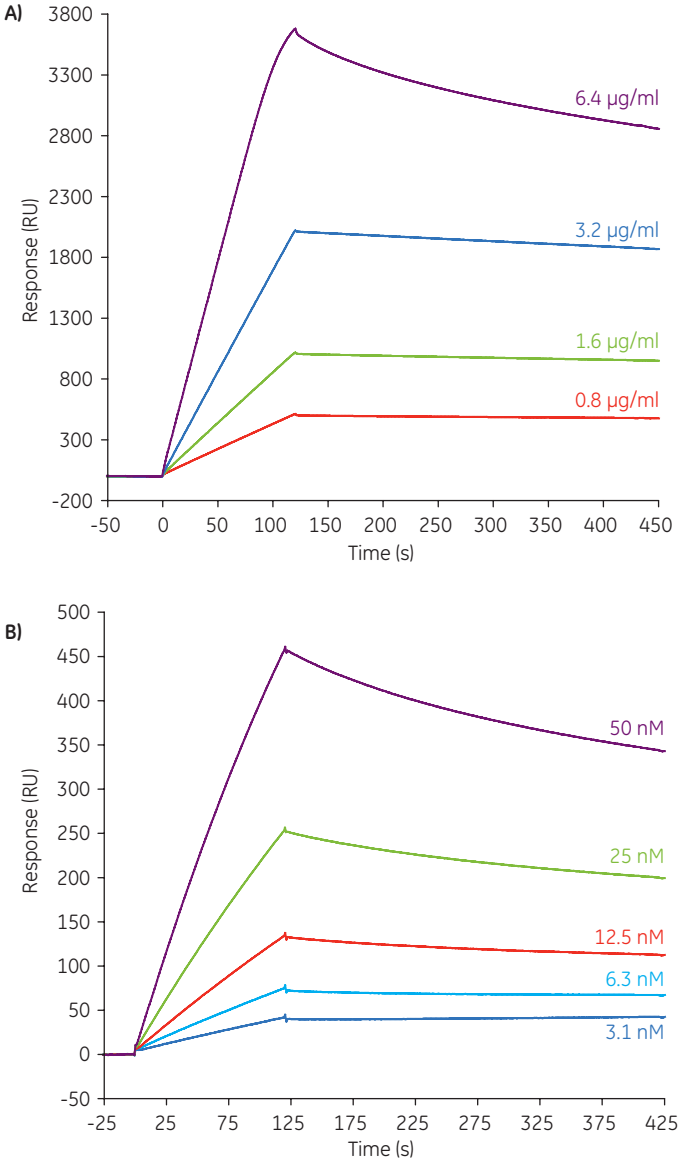


**Fig 4.** Concentration series for capture of histidine-tagged proteins over an anti-his surface: **A)** PARP10 (0.8 to 6.4 µg/ml); **B)** cystatin B (3 to 50 nM).

High-stability capture is especially critical when performing single-cycle kinetics where each analysis is performed in one long sample cycle. Kinetic characterization of the interaction between cystatin B and papain ($M_r$ 23 400) was determined using the single-cycle kinetics approach at a capture level of 30 RU, and a protein concentration of 0.125 to 2 nM on the anti-histidine surface. The results are summarized in Figure 5. The data was fitted to a 1:1 binding model with high accuracy, as demonstrated by the statistical parameters such as the reported Chi² value (0.05 RU²). The anti-histidine capture approach thus enabled a stable capture allowing confident generation of kinetic values.

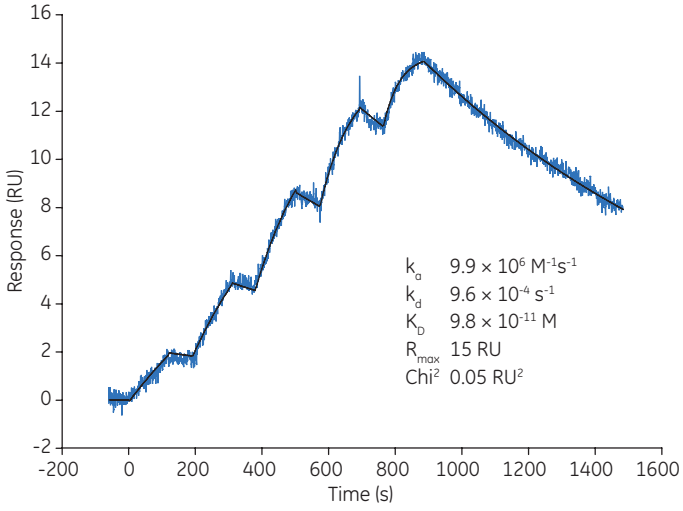


**Fig 5.** Kinetic characterization of papain (0.125 to 2 nM) binding to histidine-tagged cystatin B captured by anti-histidine antibodies immobilized to Sensor Chip CM5.

## Covalent coupling on Sensor Chip NTA

An alternative way of increasing capture stability on Sensor Chip NTA is to perform an amine coupling of the captured ligand. The NTA groups are first chelated with nickel ions and after activation with EDC/NHS, the ligand is injected and covalently bound. The nickel ions are then removed with 350 mM EDTA. This immobilization method does not require preconcentration and the ligand can be immobilized out of a crude sample. With a covalently coupled ligand, regeneration conditions must be optimized for each specific protein/analyte interaction.

Cystatin B was diluted to 6 nM in HBS-P+ buffer and immobilized on Sensor Chip NTA by the combined capture and amine coupling method. Single-cycle kinetic analysis was performed with papain as analyte (Fig 6). The data was fitted to a 1:1 binding model and the results correlated well with the kinetic constants obtained with regular capture to an anti-histidine surface (Fig 5).

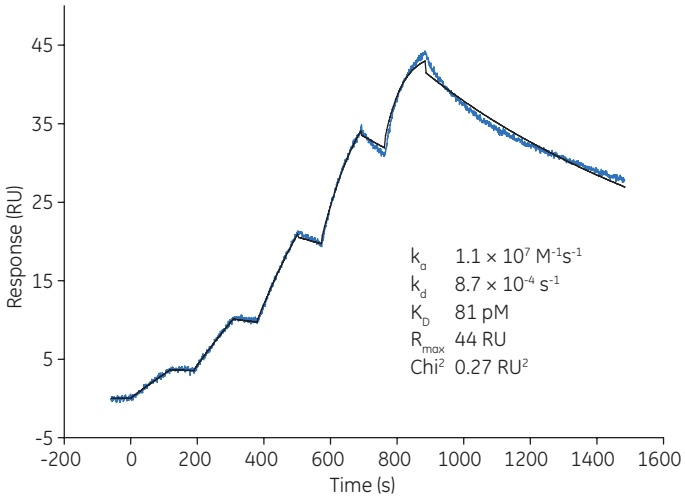


**Fig 6.** Kinetic characterization of papain (0.125 to 2 nM) binding to cystatin B covalently coupled to Sensor Chip NTA.

## Nonspecific binding

Approximately 2% of all residues in a protein are histidine. This means that, in addition to the specific tag, NTA and anti-histidine antibodies might to some extent bind to naturally occurring histidine in the proteins. Native proteins containing histidine without a histidine tag might therefore generate a response. Nonspecific binding should always be assessed in relation to the specific response to evaluate whether it will impact the results. If one of the histidine-capture approaches gives unsatisfactory results with respect to nonspecific binding, the other should be tested since the nature of nonspecific binding might differ between the two approaches.

## Tag length and composition affect capture stability

Investigations of isolated hexahistidine tags suggest that they have high affinities with a $K_D$ in the nanomolar range, and that no further improvements are seen with extended histidine tags (1). When tags are attached to proteins, however, the number of tags and the length of tags can affect capture efficiency. Currently, double histidine tags are thought to provide the best means of improving affinity on an NTA surface. Lengthening the tag does not necessarily lead to enhanced affinity as triple tags have been shown to aggregate.

In a publication from the University of York (2), capture of the protein SiaP, equipped with either of three different sets of histidine tags (hexahistidine, decahistidine, and a double tag containing both a hexahistidine and a decahistidine) immobilized on Sensor Chip NTA was studied to assess the trade-off between inadequate stability of immobilized protein at high RU levels and insufficient analyte binding response at low RUs. The results show that SiaP tagged with decahistidine was more stably captured on Sensor Chip NTA than SiaP tagged with hexahistidine, and that the best results were obtained with the double histidine tag (Fig 7).

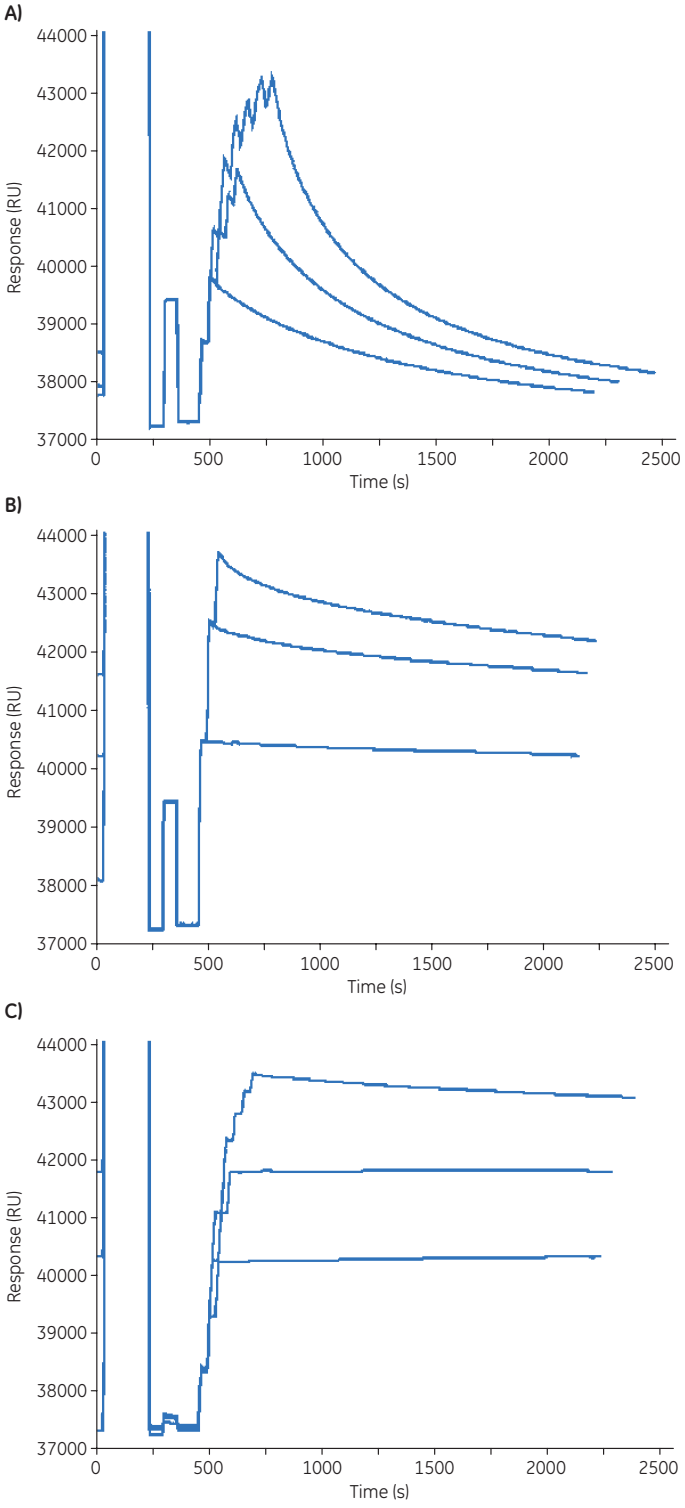


**Fig 7.** Stability of histidine-tagged SiaP on Sensor Chip NTA: **A)** hexa, **B)** deca, and **C)** double histidine-tagged protein immobilized on Sensor Chip NTA at three concentrations. Reprinted with permission from Analytical Chemistry, © 2011 American Chemical Society.

The hexa-spacer-deca histidine tag, introduced by Fischer *et al.* (3) appears to be an improved construct amongst other double histidine tags documented in literature. The long-term performance of the construct was tested in a Biacore system. Buffer was run over the surface for two days and the average baseline drift was only 2 RU/min, which is sufficiently low to be corrected for within a small-molecule binding experiment.

In order to minimize a possible negative effect on the function of the protein, it is advisable to use the smallest number of histidine residues required for efficient purification. In general, a hexahistidine tag is an appropriate choice for a first trial when incorporating a polyhistidine tag into a protein.

The final choice of construct is subject to availability of different versions of the protein and the application it will be used for; short-term or long-term screening of weakly or tightly binding analytes.

## Conclusions

Efficient capture of histidine-tagged proteins on Biacore sensor surfaces can be performed using either His Capture Kit or Sensor Chip NTA. Both alternatives give good binding stability and low nonspecific binding, and the choice is dependent on the application. For studies of low-molecular weight analytes, though, Sensor Chip NTA is the first choice. Capture capacity is high and if stability is an issue, the captured ligand can be covalently coupled. This is often an advantageous alternative since regeneration is rarely required for small molecules.

## Acknowledgements

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## References

1. Knecht, S. *et al.* Oligohis-tags: mechanisms of binding to $Ni^{2+}$-NTA surfaces. *J. Mol. Recognit.* **22** 270–279 (2009).
2. Khan, F. *et al.* Double-hexahistidine tag with high-affinity binding for protein immobilization, purification, and detection on Ni-nitrilotriacetic acid surfaces. *Anal. Chem.* **78** 3072–3079 (2006).
3. Fischer M. *et al.* Comparative assessment of different histidine-tags for immobilization of protein onto surface plasmon resonance sensorchips. *Anal. Chem.* **83** 1800–1807 (2011).

# Ordering information

| Product | Code number |
|---|---|
| His Capture Kit* | 28-9950-56 |
| Sensor Chip NTA, pack of 1 | BR-1004-07 |
| Sensor Chip NTA, pack of 3 | BR-1000-34 |
| Series S Sensor Chip NTA, pack of 1 | 28-9949-51 |
| Series S Sensor Chip NTA, pack of 3 | BR-1005-32 |
| NTA Reagent Kit† | 28-9950-43 |

* includes anti-histidine antibody and immobilization buffer for 10 immobilizations and regeneration solution for 1000 regenerations.

† includes $Ni^{2+}$ solution and regeneration solution for 1200 injections.

| Related literature | Code number |
|---|---|
| His Capture Kit, Data file | 29-0079-30 |
| Sensor Chip NTA, Data file | 29-0079-27 |

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