

Sensor Chip NTA

NTA Reagent Kit

Sensor Chip NTA and NTA Reagent Kit are designed for capture of histidine-tagged recombinant proteins in Biacore systems. Capture is an attractive alternative for protein attachment since physiological conditions can be used during the procedure. In addition, capture generates a directed structural orientation of the protein on the surface, potentially offering optimal site exposure.

Sensor Chip NTA is designed for capture of histidine-tagged proteins on nickel (Ni^{2+}) chelated nitrilotriacetic (NTA) groups for subsequent biomolecular interaction analysis. NTA Reagent Kit provides ready-to-use reagents required for the use of Sensor Chip NTA.

Sensor Chip NTA and NTA Reagent Kit deliver:

- Maximum convenience: Sensor Chip NTA is ready for nickel loading and capture.
- Ready-to-use: NTA Reagent Kit provides the nickel ion and regeneration solutions for use with Sensor Chip NTA
- Full confidence: Verified for use with Biacore systems

Attachment by capture has several advantages over covalent immobilization. Tagged proteins can be captured from crude media solutions, requiring little or no sample preparation. In addition, regeneration conditions are generic and therefore 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 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. GE Healthcare offers products for both approaches, Sensor Chip NTA with NTA Reagent Kit, and His Capture Kit (see Data file 29-0079-30). The choice between these alternatives often depends on the application. Sensor



Fig 1. Sensor Chip NTA, Series S Sensor Chip NTA, and NTA Reagent Kit for capture of histidine-tagged molecules.

Chip NTA can be used for many different types of histidine-tagged proteins and a wide range of analyte molecules can be studied, from low-molecular weight compounds to large proteins. For experiments where low molecular weight analytes are studied, 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.

Description

Sensor Chip NTA consists of carboxymethylated dextran with covalently immobilized NTA. The NTA molecule chelates metal ions such as Ni^{2+} , creating coordination sites that bind to polyhistidine tags. To allow capture of histidine-tagged proteins, the surface of Sensor Chip NTA is first activated with Ni^{2+} ions. Thereafter the analyte of interest is passed over the surface and any binding is detected. The NTA surface is then regenerated with EDTA solution after which it is ready for a new analysis cycle, starting with the Ni^{2+} solution pulse.



Regeneration may also be performed using conditions where only the analyte is removed, leaving the ligand intact on the surface for the next cycle of analyte binding. Sensor Chip NTA is compatible with a range of agents suitable for this purpose.

Sensor Chip NTA in its two formats (classic and Series S), and NTA Reagent Kit can be used in all Biacore systems except Biacore Flexchip. NTA Reagent Kit contains solutions for approximately 1200 injections.

Capture stability

There are several ways to increase the capture stability for histidine-tagged proteins. An efficient and simple approach is to decrease the protein load (Fig 2). This provides a larger number of unoccupied binding sites on the surface which, in turn, increases the probability for rebinding of dissociating proteins. Lower capture levels can be obtained by decreasing the protein concentration or by shortening the contact time.

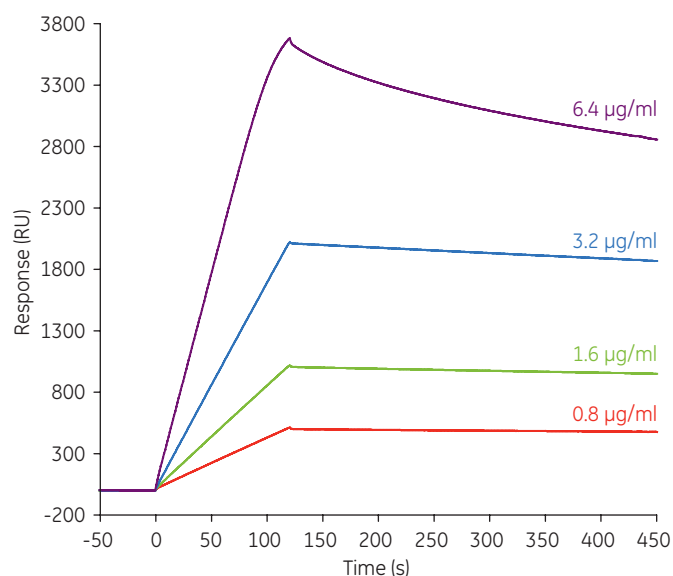


Fig 2. Injections of different concentrations of histidine-tagged green fluorescent protein (0.8 to 6.4 µg/ml) over Sensor Chip NTA.

Application

The applicability of Sensor Chip NTA is very broad. It can be used for all types of Biacore analyses, from binding studies and screening to concentration determinations and kinetic characterization. Sensor Chip NTA 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.

Kinetic analysis of the interaction between histidine-tagged tankyrase 1 captured on Sensor Chip NTA and the compound XAV939 (M_r 310) was run in Biacore T200. The results are shown in Figure 3. Tankyrase 1 is a M_r 29 000 protein belonging to the poly-(ADP-ribose)-polymerase (PARP) family, a group of proteins gaining increased interest as targets for drug discovery. Concentrations between 0.06 µM and 5 µM were used. Data were fitted to a 1:1 binding model with high accuracy, as demonstrated by statistical parameters such as the reported χ^2 value (0.04 RU²). This confirms the accuracy of the kinetic determination. Sensor Chip NTA thus enabled a stable capture allowing confident generation of kinetic values.

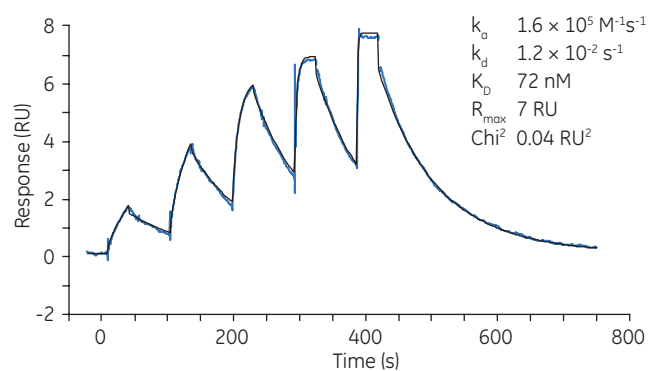


Fig 3. Kinetic characterization of compound XAV939 binding to tankyrase1 (760 RU) captured onto Sensor Chip NTA.

Acknowledgements

Tankyrase 1 was kindly provided by the Structural Genomics Consortium, Karolinska Institutet, Sweden.

Ordering information

Product	Quantity	Code number
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 for Biacore Flexchip

Related products

His Capture Kit	1	28-9950-56
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Related literature

Approaches for capture of histidine-tagged proteins in Biacore, White paper	29-0079-29
His Capture Kit, Data file	29-0079-30
Sensor Surface Handbook	BR-1005-71

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First published Oct. 2011

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