User guide

NTA modified Sensor chips &

His-tag capture Kit

Product list

Sensor chip lists

Lists	ea/pkg	Product #	Recommended Analytes / Storage temperature
NTA-Au chip	10	PCNT1000	Proteins, Vesicles, Nano-particles, Cells, Peptides, Oligomers Storage: 2~8℃
NiHC1000	3	HCNT101KX	Small molecules Storage: -20℃

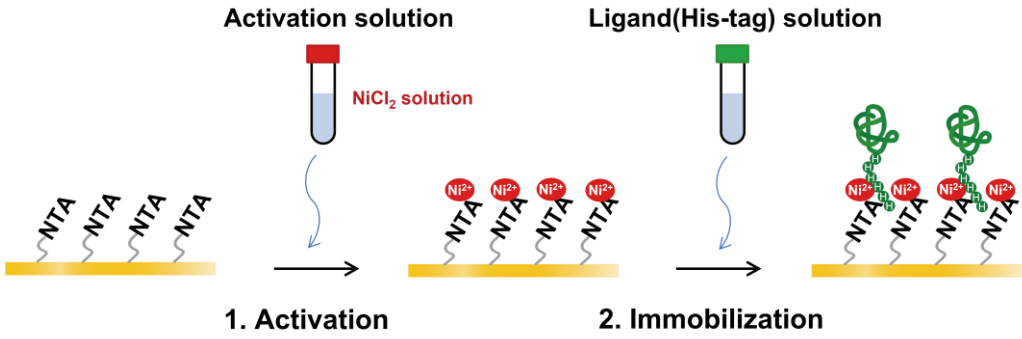
Buffers & Reagents

Lists	volume/pkg	Product #	Purpose of use
HBST buffer (10x)	50ml	RBHT1010-50	Running buffer, equilibrium, washing, dissociation
His-tag capture kit	100 tests	IMHT1000	Ligand immobilization

Note: For research only use

Histag-Protein capture

Recombinant proteins often have his-tags. If the protein is not immobilized well by the amine coupling method or the binding signal is weak, a method of non-covalently immobilizing the his-tag site to the NTA sensor chip can be selected. This method is useful when the ligand protein is diverse because the ligand protein is also desorbed during the regeneration step. For the same reason, the single cycle kinetics method is recommended for kinetics evaluation. Since the bond between NTA and 6xhistidine is not very strong, it may be continuously desorbed during the experiment. It is recommended to proceed with the analysis when drift is minimized after sufficient washing. The NiHC1000 sensor chip provided by iclubio is a special product that strongly improves the binding force between NTA and 6xhistidine. If you are concerned about the above issues, we recommend using the NiHC1000.



His-tag capture Kit lists

Products #	Including
IMHT1000	350mM EDTA solution, 50 ml 5mM $NiCl_2$ solution, 25 ml 4M Imidazole, 25 ml 10XHBST, 50 ml

His-tag capture Kit: Preparation and Storage

Contents	Aliquots and storage
$NiCl_2$ solution	1. Aliquots in 250 ul each 2. Storage: -20℃
EDTA solution, 50 ml	Storage: room temperature
4M Imidazole, 50 ml	Storage: room temperature
10X HBST, 50 ml	Storage: room temperature

Sensor chip loading & priming

1 Carefully take out the refrigerated sensor chip from the sensor chip case using a flat tweezer and mount it in the prism holder.

Note: The side that is attached to the sensor chip case is the side that connects with the prism of the prism holder. Reversing the sensor chip does not guarantee 100% performance for reuse.

2 After priming with 1X HBST buffer, wash the sensor chip with 350mM EDTA borate buffer for 3-5 minutes.

Note: Prepare 10X HBST buffer by diluting 10-fold in DIw just before the experiment.

Ligand immobilization

1 Prepare frozen $NiCl_2$ solution (250 ul) , d regeneration buffer at room temperature.

Note: Ligand materials are prepared according to material properties. After preparing at room temperature, proceed with the experiment as quickly as possible.

2 Activation: After preparing 250 ul, flow on the sensor chip for 5-10 minutes to make the NTA surface the Ni^{2+} surface only ligand channel (channel 1).

Note: The recommended flow rate for the activation step is 10-30 ul/min.

3 Ligand immobilization: Dilute ligand in acetate buffer to a concentration of 1 ~ 100 ug/ml and inject for 1 ~ 30 minutes.

Note: The immobilization step is performed by adjusting the time, flow rate, and concentration according to your target immobilization level.

Note: If there is a lot of nonspecific adsorption as a result of binding to analyte, prepare serum albumin at 100 ug/ml and perform additionally after ligand immobilization.

Analytes interaction

1 If no more desorbed signals are observed and sensorgram equilibrium is observed, analyze analyte binding. First, inject 1 to 3 representative concentrations (100 nM, 1 uM, 10 uM) to check if there is a signal.

Note: For chemical, it is recommended to perform up to 100 uM.

2 If a signal is detected, dilute by 2 times from the concentration determined to be the saturation concentration, and obtain 5 or more sensorgrams for each concentration. A concentration of 0 nM (running buffer) can also be used to obtain a sensorgram and correct for non-binding signals.

Note: For kinetics evaluation, it is recommended to proceed with a flow rate of 50 ~ 70 ul/min. For bonds that do not dissociate well, it is recommended that the dissociation period be 5 to 10 times longer than the association period.

Regeneration

For bonds that do not dissociate completely, a regeneration step using a regeneration buffer is required. To analyze multiple concentrations, regeneration is performed between concentrations. Since the binding between the NTA surface and the his-tag protein is non-covalent, the his-tag protein is desorbed upon regeneration. Therefore, his-tag protein must be adsorbed again after regeneration. EDTA, Imidazole, NaCl, etc. can be used as a regeneration solution.

Ligand immobilized chip storage

Regenerated NTA sensor chip from which His-tag protein has been removed can be stored using the storage kit provided by iclubio. After attaching the dedicated storage FM to the prism holder, load the glycerol buffer. It depends on the characteristics of the ligand, but it can be used after storage for at least 1 month.

Note: For more detailed instructions, please refer to the handbook provided when purchasing the device.

www.icluebio.com

icluebio's sensor chips, buffers, and reagents are manufactured in Korea, and are finally delivered to customers through precise quality inspection by a specialist.

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