heliX+
User Manual

BRUKER
Biosensors

NEW Y-STRUCTURE HIS CAPTURE KIT

for capture strategy on either the red or green arm of the new **Y-structure** design

Dynamic Biosensors GmbH
HK-NYS-NTA v1.0

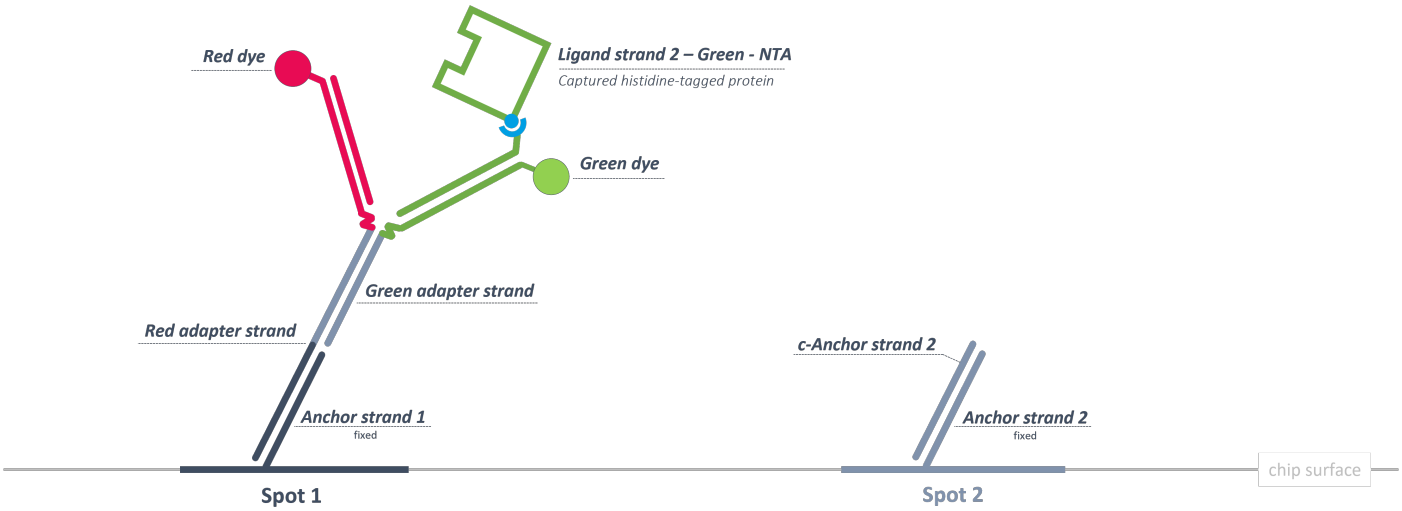
Key Features

- This kit is designed for capture of **histidine-tagged proteins** (His6 or His10) using **Tris-NTA**.
- Includes ***Ligand strand 1*** - **Red** and ***Ligand strand 2*** - **Green** with Tris-NTA for **20 regenerations** each.
- For the new **Y-structure** kit only.
- It is a possibility for dimerization projects.
- Homo-/hetero-proteins can be coupled easily to the arms via his-tag capture.
- Compatible with **heliX® Adapter Chip**, both **Spot 1** and **Spot 2**

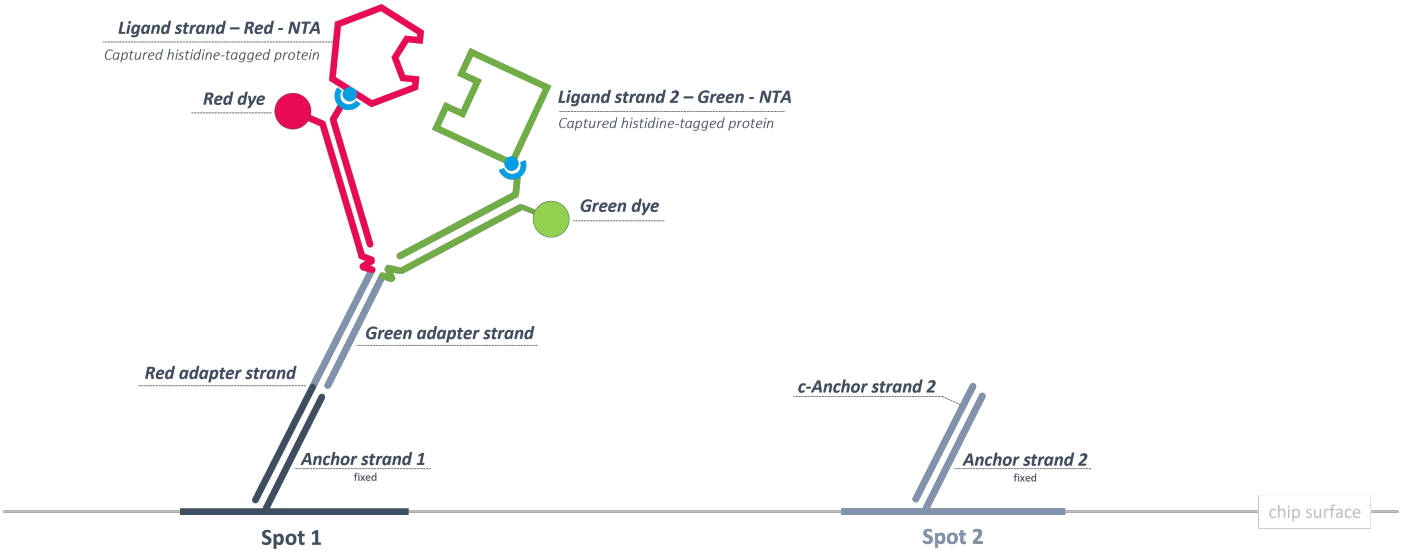
heliX® Adapter Chip Overview

2 spots with 2 different anchor sequences for DNA-encoded addressing. Spot 1 is functionalized with the capture molecule while Spot 2 is used as real-time reference.

Single Capture



Double Capture



Product Description

Order Number: **HK-NYS-NTA**

Table 1. Contents and Storage Information

Material	Cap	Concentration	Amount	Buffer	Storage
New **Y-structure *Red Adapter strand - NTA***	Purple	500 nM	2 x 100 µL	TE40 [1]	-20°C
New **Y-structure *Green Adapter strand - NTA***	Purple	500 nM	2 x 100 µL	TE40 [1]	-20°C
Loading Solution ($NiCl_2$)	Transparent	10 mM	10 x 1500 µL	TE40 [1]	-20°C
Imidazole Solution	Transparent	250 mM	10 x 2000 µL	TE140 [2]	-20°C

For research use only.

This product has a limited shelf life, please see expiry date on label.
After preparation of ready to use solution the expiry date is **6 months**.

Preparation

Step 1

For surface functionalization, the **Y-structure *Red Adapter strand*** harboring the red dye **Ra** and the **Y-structure *Green Adapter strand*** harboring the green dye **Ga** need to be pre-hybridized with either the new **Y-structure *Red Adapter strand - NTA*** or new **Y-structure *Green Adapter strand - NTA*** and a conjugated ***Ligand strand***.

Example. In-solution hybridization of **Y-structure** strands with a combination of covalent coupling protein in green & his-tag capture strategy in red:

i. Mix the following components:
   1. New **Y-structure** Red Adapter strand with Ra (400 nM)
   2. New **Y-structure** Green Adapter strand with Ga (400 nM)
   3. New **Y-structure** Red Adapter strand - NTA (500 nM)
   4. Covalently conjugated protein to **Y-structure *Ligand strand 2*** - Green (500 nM)

   Combine at a 1:1 ratio (v/v).

ii. Incubate the solution of step i) at RT for at least **2 hours** to ensure complete hybridization. Overnight incubation at 4°C is also possible.

Step 2

Mix solution of step ii) and **cAnchor strand 2** (100 nM) at 1:1 ratio (v/v).

Step 3

Solution is ready to use for **heliX® Adapter Chip** functionalization.

Example

Required volume for one functionalization for a combination of amine coupling and capture on the new **Y-structure**: **35 µL** with a final concentration of **50 nM**.

Vial 1				Vial 2
New **Y-structure** Red Adapter strand with Ra (400 nM)	New **Y-structure** Green Adapter strand with Ga (400 nM)	**Y-structure** Red Adapter strand - NTA (500 nM)	Conjugated protein to **Y-structure *Ligand strand 2*** (500 nM)	**cAnchor strand 2** (100 nM)
4.5 µL	4.5 µL	4.5 µL	4.5 µL	18 µL

Assay Setup in heliOS

This specific kit requires a customized method consisting in **His-tag capture** plus **Y-Structure FRET Kinetics**, which is currently not provided among the verified assay. It can be easily created by an advanced **heliOS** user by applying the default parameters already existing in the two different and separate workflows (please refer to the **heliX**$^{+}$ guide available at this **link**); however, for any help on creating the new method, please contact the support team at **support.dbs@bruker.com**.

TIP | *As the stability of his capture is affected by the protein, in case of long dissociations, consider using the classic conjugation approach.*

Useful Order Numbers

Table 2. Order Numbers

Product Name	Comment	Order No
heliX® **Adapter Chip**	Chip with 2 detection spots	ADP-48-2-0
Y-structure Amine coupling kit 1 - **Red**	3 conjugations	HK-NYS-NHS-1
Y-structure Amine coupling kit 2 - **Green**	3 conjugations	HK-NYS-NHS-2
New Y-structure Kit 1: for proximity binding assay **Spot 1**	400 nM x 250 µL	HK-NYS-1
New Y-structure Kit 2: for proximity binding assay **Spot 2**	400 nM x 250 µL	HK-NYS-2

Contact

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www.dynamic-biosensors.com

Instruments and chips are engineered and manufactured in Germany.

For Research Use Only. Not for use in clinical diagnostic procedures.

[**1**] TE40: 10 mM Tris, 40 mM NaCl, 0.05 % Tween20, 50 µM EDTA, 50 µM EGTA
[**2**] TE140: 10 mM Tris, 40 mM NaCl, 0.05 % Tween20, 50 µM EDTA, 50 µM EGTA