heliX+
User Manual

BRUKER
Biosensors

NEW Y-STRUCTURE KIT 2

for proximity binding assay - **Spot 2**

Dynamic Biosensors GmbH
HK-NYS-2 v1.0

Key Features

- Ideal for studying **ternary complex formation** upon binding of bispecific small molecules (e.g., **PROTACs**, molecular glues), homo-dimerization and bispecific antibodies with weak affinities.
- The new optimized **Y-structure** design allows higher **FRET** sensitivity.
- Compatible with **heliX® Adapter Chip**.
- The **Y-structure Red Adapter** strand (specific to Spot 2) carries a moderately hydrophilic red dye (**Ra**) with a single positive net charge.
- The **Y-structure Green Adapter** strand carries a hydrophilic green dye (**Ga**) with a single negative net charge.
- Green and red dyes can detect binding on each arm via **Fluorescence Proximity Sensing (FPS)**, or report if the structure is OPEN or CLOSED via sensitive **FRET**.
- Homo-/hetero-proteins can be coupled easily to the arms via exchangeable ligand strands.
- The flexible hinge region confines two proteins to a small volume and defined distance.

Product Description

Order Number: **HK-NYS-2**

Table 1. Contents and Storage Information

Material	Cap	Concentration	Amount	Buffer	Storage
New **Y-structure** ***Red Adapter strand 2 with Ra***	Orange	400 nM	5 x 50 µL	TE40 [1]	-20°C
New **Y-structure** ***Green Adapter strand with Ga***	Green	400 nM	5 x 50 µL	TE40 [1]	-20°C
New **Y-structure** ***Ligand Free Strand (lfs) - Red*** for binary binding in green	Yellow	500 nM	3 x 100 µL	TE40 [1]	-20°C
New **Y-structure** ***Ligand Free Strand (lfs) - Green*** for binary binding in red	Yellow	500 nM	3 x 100 µL	TE40 [1]	-20°C

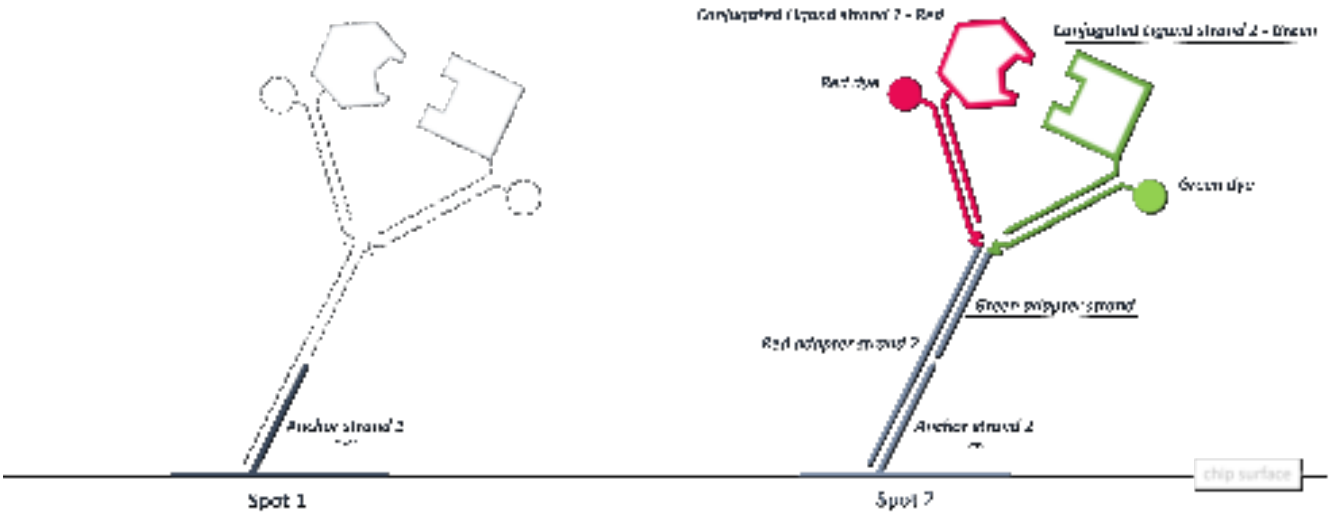
For research use only.

This product has a limited shelf life, please see expiry date on label.

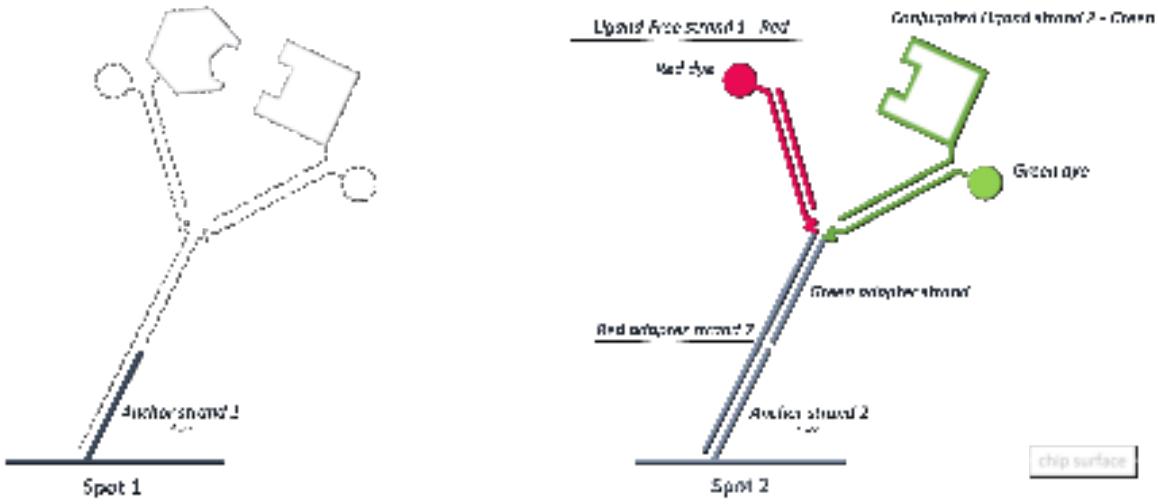
To avoid many freeze thaw cycles please aliquot the nanolever.

heliX® Adapter Chip Overview

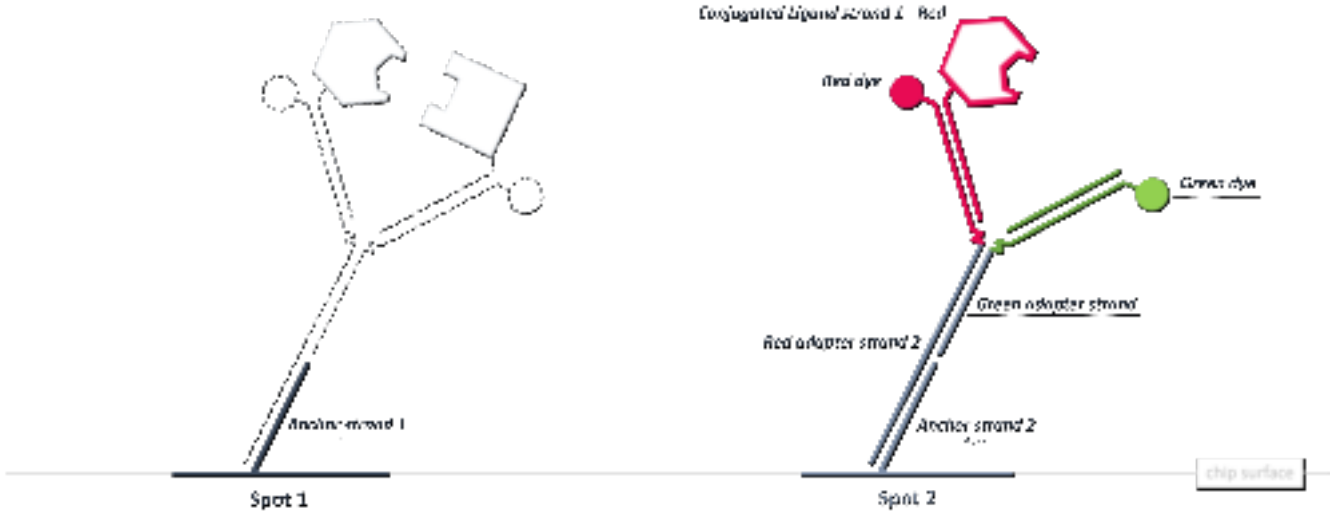
Ternary binding



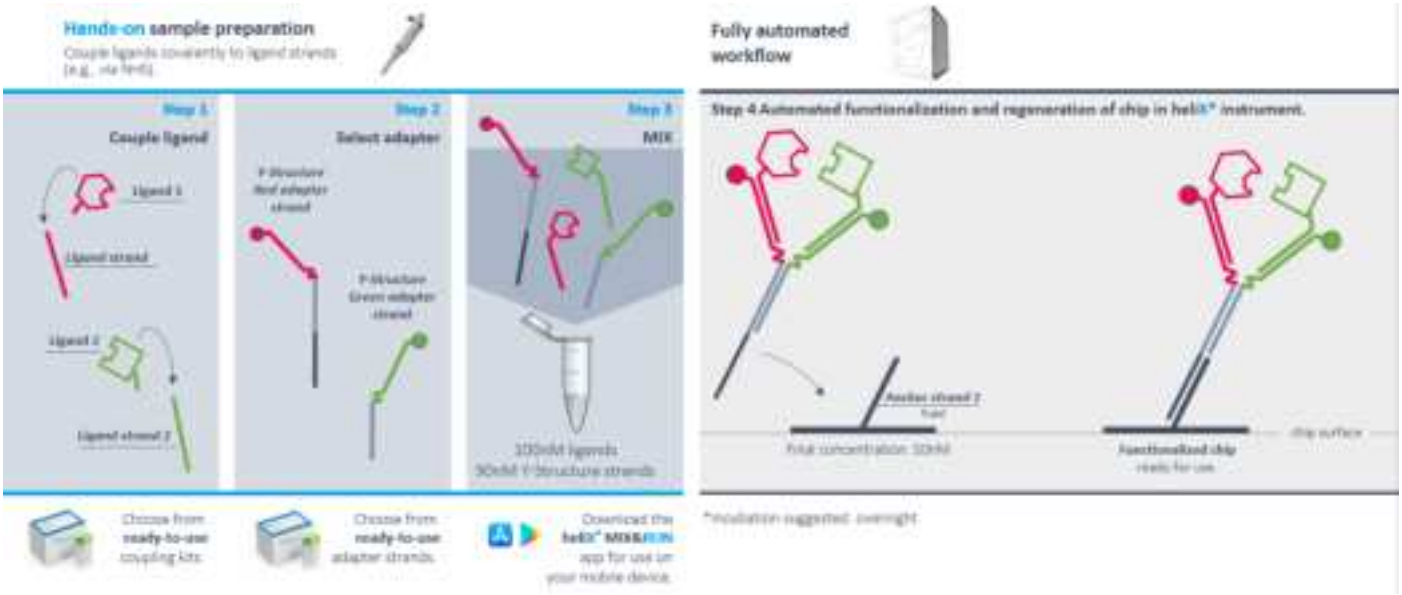
Binary binding in green



Binary binding in red



Preparation



Step 1

The *Ligand strands* can be extended at either the 5' (***Ligand strand 1*** - Red arm) or 3' (***Ligand strand 2*** - Green arm) end with any DNA/RNA sequence. Additionally, they can be crosslinked to a protein of interest through amine coupling using the specialized **heliX**® Amine Coupling Kits (**HK-NYS-NHS-1**, which hybridizes to the red arm, and **HK-NYS-NHS-2**, which hybridizes to the green arm). It is highly recommended to purify the conjugation product with **pro**FIRE® before conducting kinetic studies.

TIP	*For higher FRET quality, the covalent coupling of the ligands is recommended. + Alternatively, the protein can be captured via His-tag using the HK-NYS-NTA kit.*

Step 2

For surface functionalization, the **Y-structure *Red Adapter strand 2*** harboring the red dye **Ra** and the **Y-structure *Green Adapter strand*** harboring the green dye **Ga** need to be pre-hybridized with the ***Ligand-strands*** in order to build the **Y-structure**.

Example of sample preparation for measuring **ternary binding on Spot 1** and **binary binding in green on Spot 2**:

Vial 1 | In solution hybridization of **Y-structure** strands and ligand strands for **Spot 1** (*not included in this kit, refer to HK-NYS-1, HK-NYS-NHS-1 and HK-NYS-NHS-2*).

i. Mix **Y-structure *Red Adapter strand*** (400 nM), ***Green Adapter strand*** (400 nM) conjugated ***Ligand strand 1*** (500 nM) and conjugated ***Ligand strand 2*** (500 nM) at a 1:1 ratio (v/v).
ii. Incubate the solution of step i) at **RT for 2 hours** to ensure complete hybridization. Overnight incubation at 4°C is also possible, but it depends on the stability of the conjugated protein.

Vial 2 | In solution hybridization of **Y-structure** strands and ligand strands for **Spot 2**.

i. Mix **Y-structure *Red Adapter strand 2*** (400 nM), ***Green Adapter strand*** (400 nM), ***Ligand-free strand 1 - Red*** (500 nM) and conjugated ***Ligand strand 2*** - ***Green*** (500 nM) at a 1:1 ratio (v/v).
ii. Incubate the solution of step i) at **RT for 2 hours** to ensure complete hybridization. Overnight incubation at 4°C is also possible, but it depends on the stability of the conjugated protein.

Step 3

Mix solution of step 2 at 1:1 ratio (v/v).

Step 4

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

Example

Required volume for 1 functionalization: **35 µL** with a final concentration of **50 nM**.

Vial 1		Vial 2	
New **Y-structure** ***Red Adapter strand & Green Adapter strand*** (400 nM)	Conjugated ***Ligand strand 1*** & Conjugated ***Ligand strand 2*** (500 nM)	New **Y-structure** ***Red Adapter strand 2 & Green Adapter strand*** (400 nM)	***Ligand-free strand 1 - Red*** & Conjugated ***Ligand strand 2*** (500 nM)
4.5 µL each > 9 µL tot	4.5 µL each > 9 µL tot	4.5 µL each > 9 µL tot	4.5 µL each > 9 µL tot

After incubation time, mix vial 1 and vial 2 to obtain a ready-to-use DNA solution.

Assay Setup in heliOS

For studying **ternary complex formation** *upon binding of bispecific small molecules (e.g.,* **PROTACs***, molecular glues).*

Go to **heliOS** > create a **New Assay Workflow** > add **Custom Assay** > load **Y-Structure FRET Kinetics** > modify the parameters based on your needs and run the assay.

Suggested assay parameters (e.g., flow rates, functionalization time, LED power, etc.) are within the **heliOS** assay.

IMPORTANT | *For binary interaction in red, please set LED red ≥ 1. However, do not forget to set it back to 0 when* **FRET** *interaction are under investigation.*
For more details, please refer to the **heliX**⁺ *guide available this* **link**.

Alternatevely, **Y-Structure FRET Kinetics - auto LED** assay can be utilized, where the **heliX®** system automatically adjusts the LED power to optimize the fluorescence signal for a better signal-to-noise ratio. This approach is highly recommended for weak binders or screening applications.

For studying bispecific antibodies with weak affinities (e.g., Hemlibra binding to Factor X and IX)

Go to **heliOS** > create a **New Assay Workflow** > add simply **Kinetics with Functionalization** from the **Custom Assay** list > modify the parameters based on your needs and run the assay.

TIP | *Antibodies are big proteins which do not allow to bring the two dyes in close proximity, therefore* **FRET** *cannot be recorded. This is the reason why classic kinetics workflow and* **Fluorescence Proximity Sensing (FPS)** *is used for detecting binding.*

For further questions, please contact the support team at **support.dbs@bruker.com**.

Useful Order Numbers

Table 2. Order Numbers

Product Name	Amount	Order No
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 His Capture Kit	500 nM x 200 µL	HK-NYS-NTA

Contact

Dynamic Biosensors GmbH
Perchtinger Str. 8/10
81379 Munich
Germany

Bruker Scientific LLC
40 Manning Road, Manning Park
Billerica, MA 01821
USA

Order Information **order.biosensors@bruker.com**
Technical Support **support.dbs@bruker.com**

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