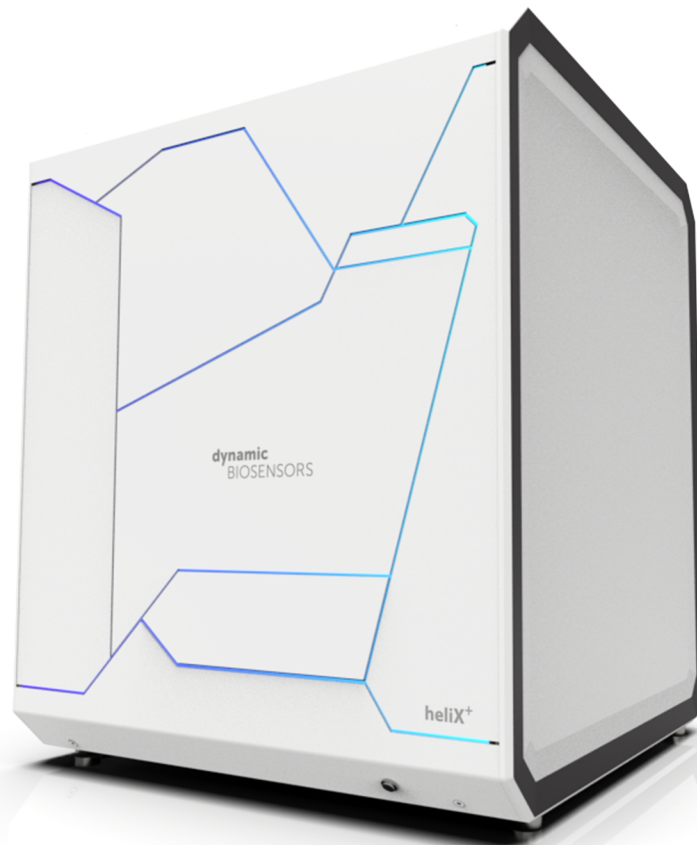


Y-STRUCTURE KIT 2

for proximity binding assay - **Spot 2**

Dynamic Biosensors GmbH
HK-YS-2 v1.1



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.
- In combination with **Y-structure Kit 1**, this kit is ideal for studying **simultaneously ternary vs binary binding affinities**.
- Compatible with **heliX[®] Adapter Chip**.
- The **Y-structure Red Adapter strand 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-YS-2**

Table 1. Contents and Storage Information

Material	Cap	Concentration	Amount	Buffer	Storage
Y-Structure Red Adapter strand 2 with Ra	Red	400 nM	5 x 50 µL	TE40 ^[1]	-20°C
Y-Structure Green Adapter strand with Ga	Green	400 nM	5 x 50 µL	TE40 ^[1]	-20°C
Ligand-free strand for binary binding in green	Yellow	500 nM	3 x 100 µL	TE40 ^[1]	-20°C
Ligand-free strand 2 for binary binding in red	Yellow	500 nM	3 x 100 µL	TE40 ^[1]	-20°C

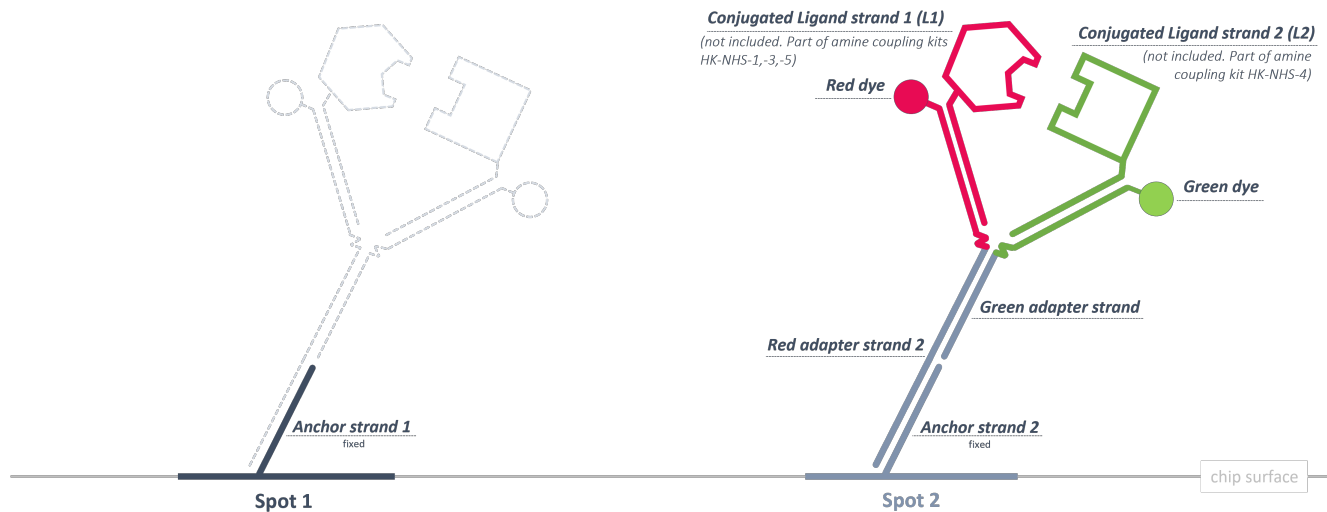
For research use only.

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

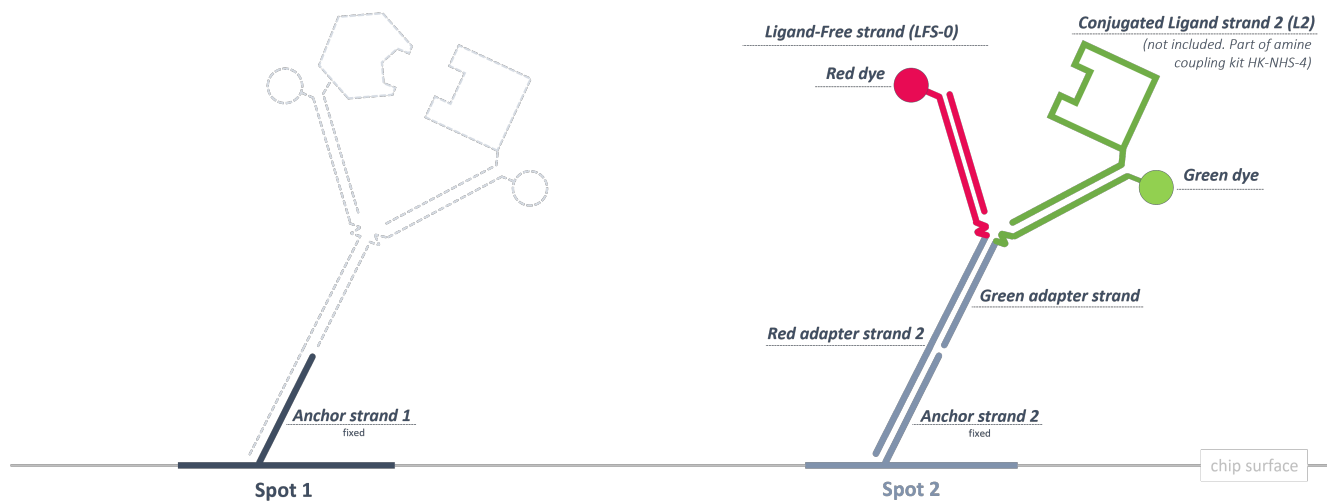
To avoid many freeze thaw cycles please aliquot the nanolever.

heliX[®] Adapter Chip Overview

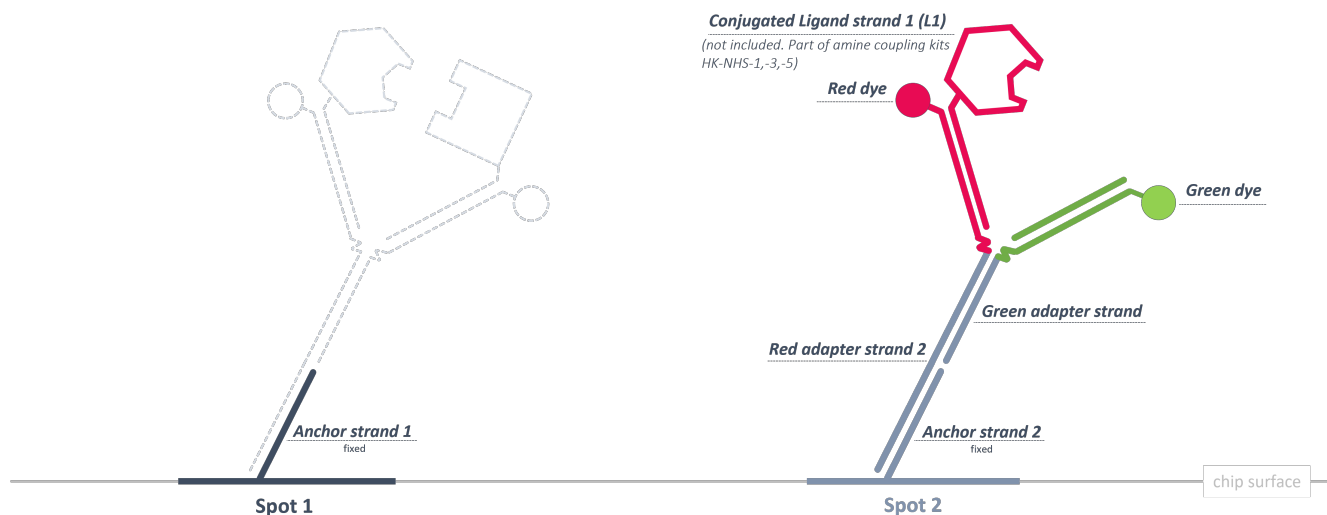
Ternary binding



Binary binding in green



Binary binding in red



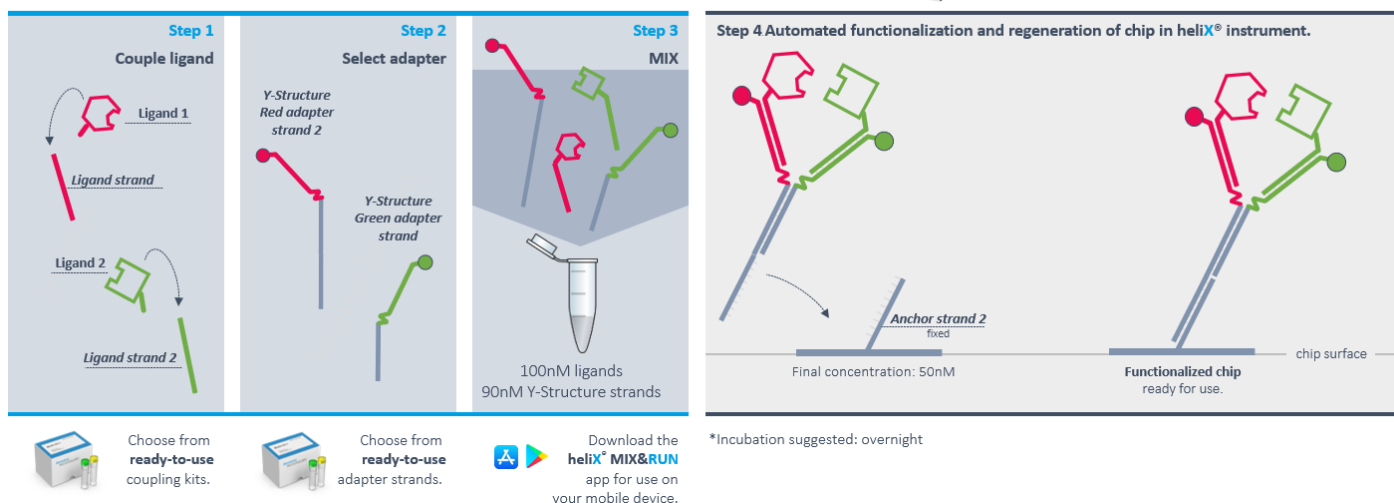
Preparation

Hands-on sample preparation

Couple ligands covalently to ligand strands (e.g., via NHS).



Fully automated workflow



Step 1

The **Ligand-strands** can be elongated at the 5' (**Ligand strand**) or 3' (**Ligand strand 2**) end with any DNA/RNA sequence, or they can be crosslinked to a protein of interest. In the latter case, the protein can be, for example, conjugated via amine-coupling using the **heliX[®]** Amine Coupling Kit 1 (**HK-NHS-1**) to the **Ligand strand** (which hybridizes to the red arm) and using the **heliX[®]** Amine Coupling Kit 4 (**HK-NHS-4**) to the **Ligand strand 2** (which hybridizes to the green arm). **heliX[®]** Amine Coupling Kits 3 and 5 (**HK-NHS-3**, **HK-NHS-5**) are also suitable for conjugation to the **Ligand strand** in case of low pl or his-tagged proteins.

In all the previous cases, we recommend to perform the purification of the conjugation product using the **proFIRE[®]**.

TIP

For higher FRET quality, the covalent coupling of the ligands is recommended. However, capture strategies can be also an option, depending on the molecules under investigation. For any questions, please contact the support team at support.dbs@bruker.com.

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, see **HK-YS-1**).

- Mix **Y-structure Red Adapter strand** (400 nM), **Green Adapter strand** (400 nM) conjugated **Ligand strand** (500 nM) and conjugated **Ligand strand 2** (500 nM) at a 1:1 ratio (v/v).
- 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, 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** (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 at least 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	
Red Adapter strand & Green Adapter strand (400 nM)	Conjugated Ligand strand & Conjugated Ligand strand 2 (500 nM)	Red Adapter strand 2 & Green Adapter strand (400 nM)	Ligand-free strand & 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.

TIP

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.

There are two ways to analyze the data: Select > Fluorescence Resonance Energy Transfer when a single analyte and multiple concentrations are measured. Select > Screening, when multiple analytes at a single concentration are investigated.

Alternatively, **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.

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Instruments and chips are engineered and manufactured in Germany.

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