heliX+
User Manual

dynamic
BIOSENSORS

# Y-STRUCTURE KIT 2

for proximity binding assay - **Spot 2**

Dynamic Biosensors GmbH & Inc.
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 |

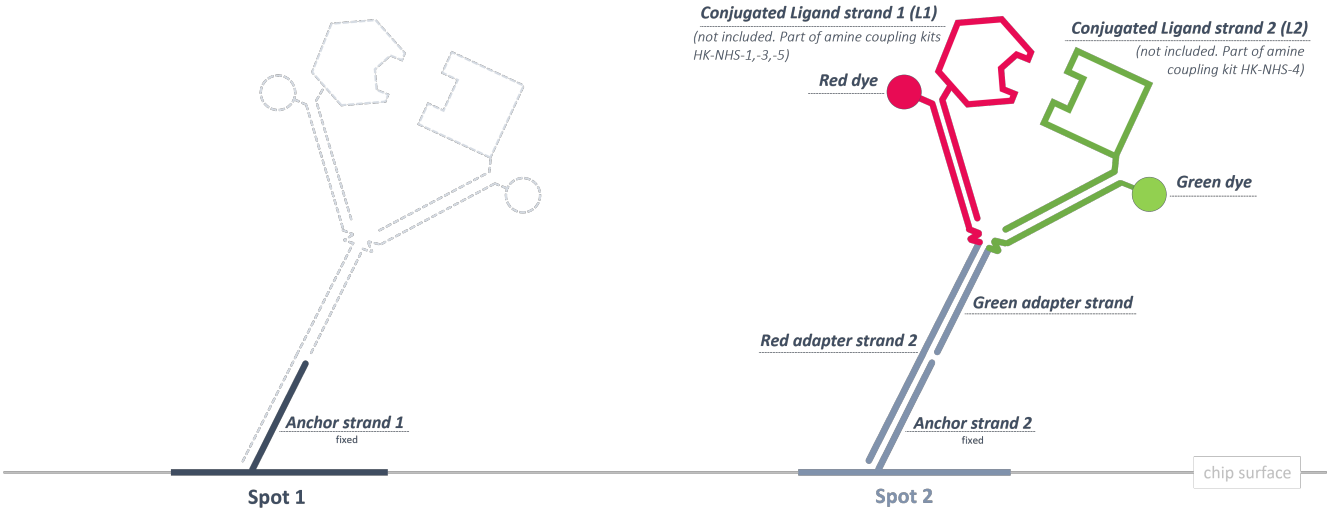
For research use only.

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

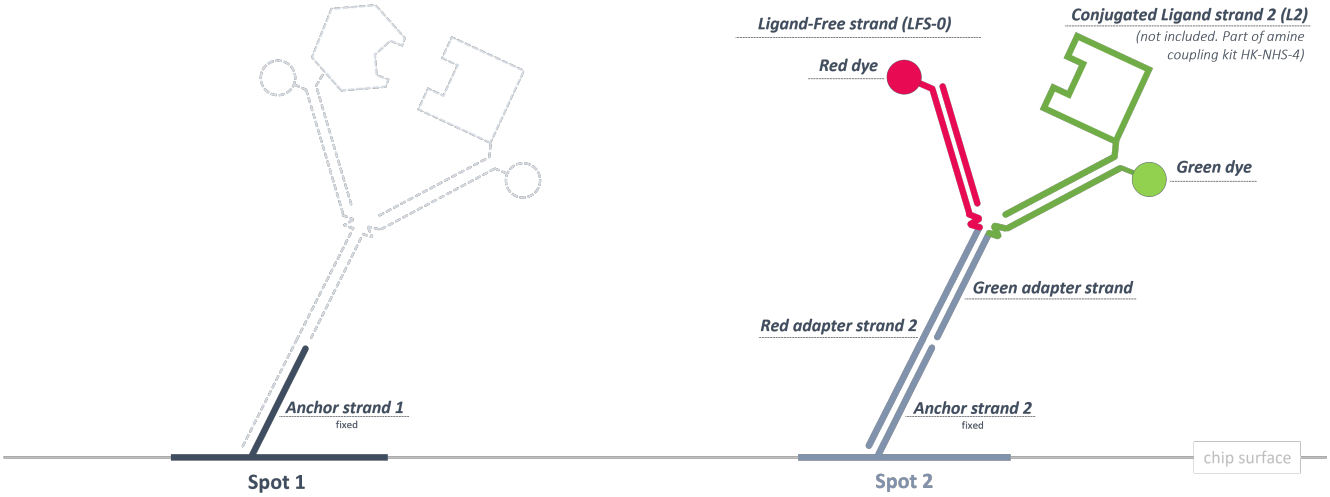
To avoid many freeze thaw cycles please aliquot the nanolever.

# heliX® Adapter Chip Overview

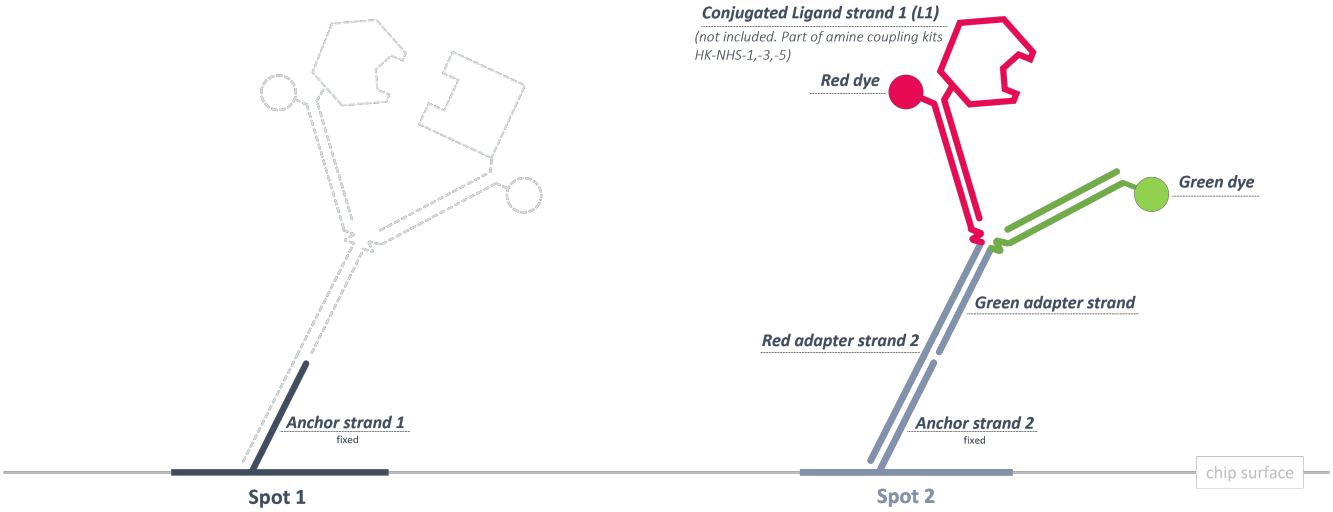
## Ternary binding



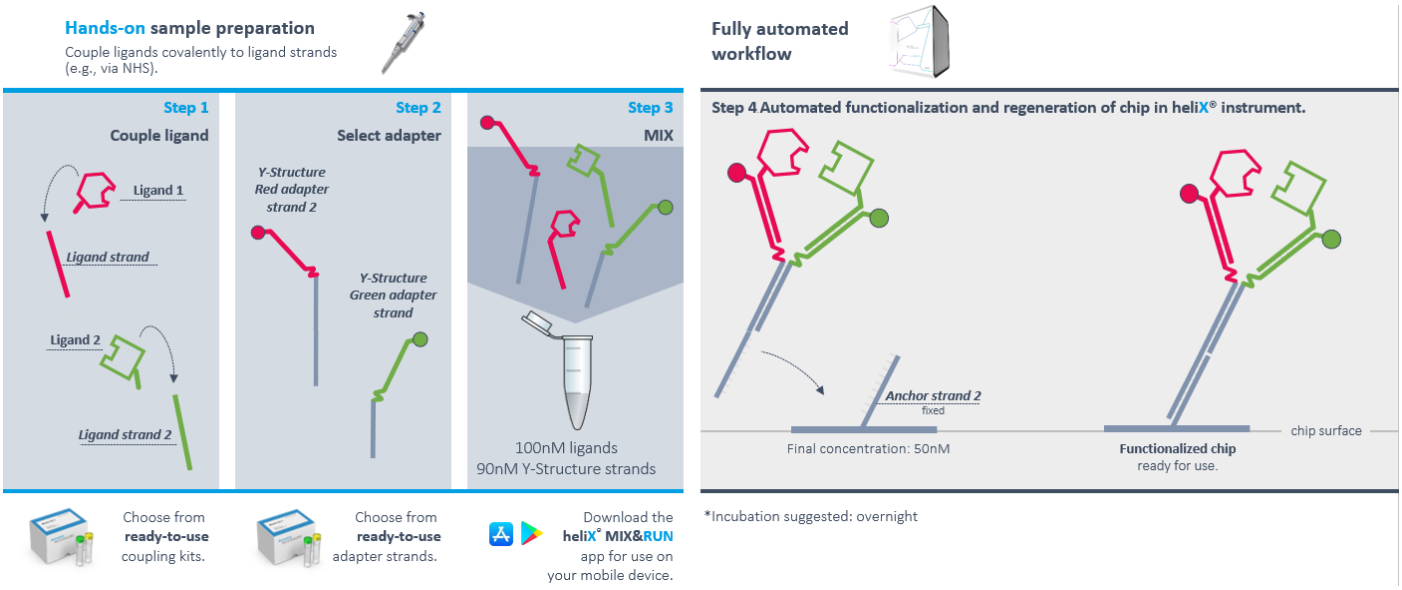

## Binary binding in green




## Binary binding in red



# Preparation




## 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 pI or his-tagged proteins.
In all the previous cases, we recommend to perform the purification of the conjugation product using the **pro**FIRE®.

**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@dynamic-biosensors.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*).

i. 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).
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.

**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.*

*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 weak binder** or **Functionalization and Kinetics** 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@dynamic-biosensors.com**.

## Contact

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Instruments and chips are engineered and manufactured in Germany.


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