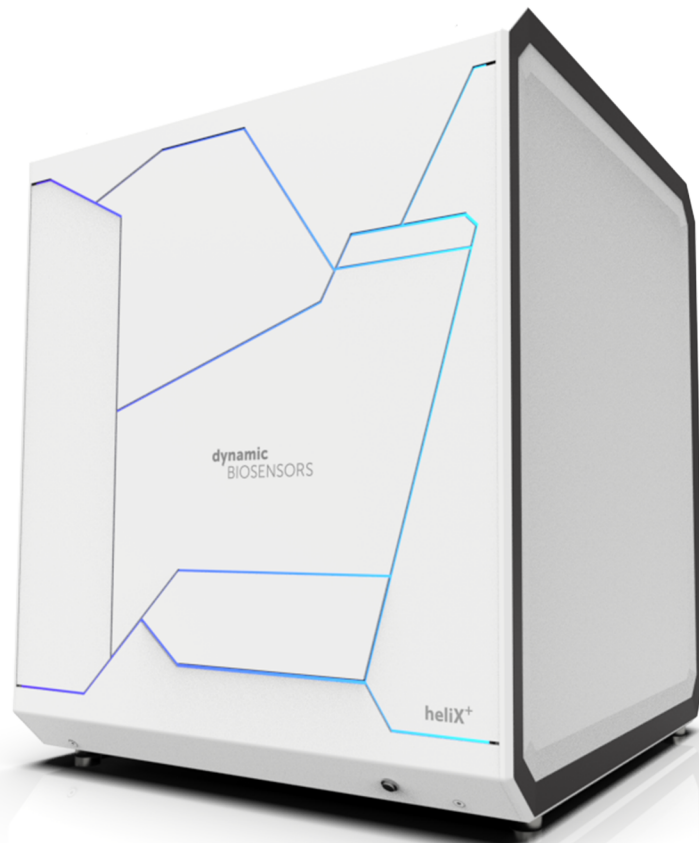# Y-STRUCTURE KIT 1
for proximity binding assay - **Spot 1**

Dynamic Biosensors GmbH & Inc.
HK-YS-1 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.

- Compatible with **heliX® Adapter Chip**.

- The **Y-structure Red Adapter** strand 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-1**

Table 1. Contents and Storage Information

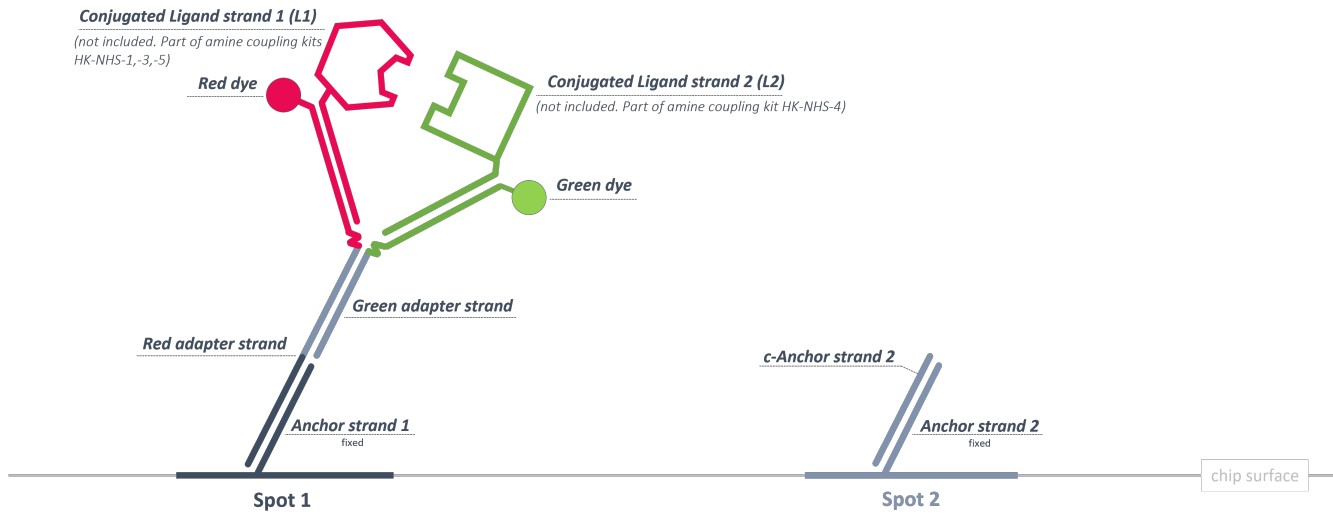| Material	Cap	Concentration	Amount	Buffer	Storage
Y-Structure Red Adapter strand with Ra	Red	400 nM	5 x 50 µL	TE40 [1]	-20°C
Y-Structure Green Adapter strand with Ga	Green	400 nM	5 x 50 µL	TE40 [1]	-20°C
Ligand-free strand for binary binding in green	Yellow	500 nM	3 x 100 µL	TE40 [1]	-20°C
Ligand-free strand 2 for binary binding in red	Yellow	500 nM	3 x 100 µL	TE40 [1]	-20°C
cAnchor strand 2	Blank	100 nM	5 x 200 µL	TE40 [1]	-20°C

For research use only.

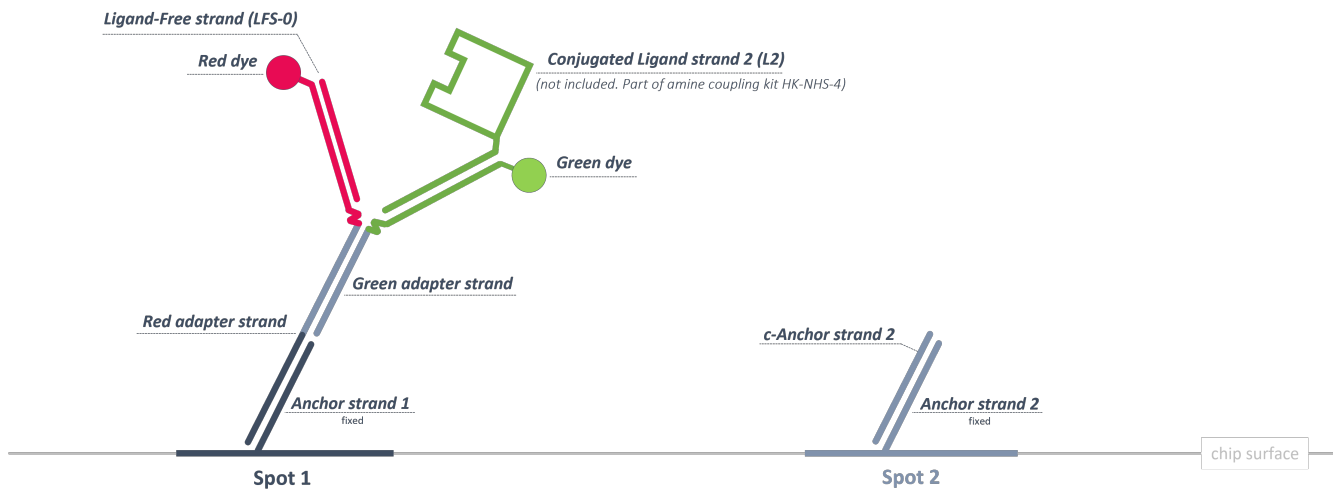This product has a limited shelf life, please see expiry date on label.

To avoid many freeze thaw cycles please aliquot the nanolever.
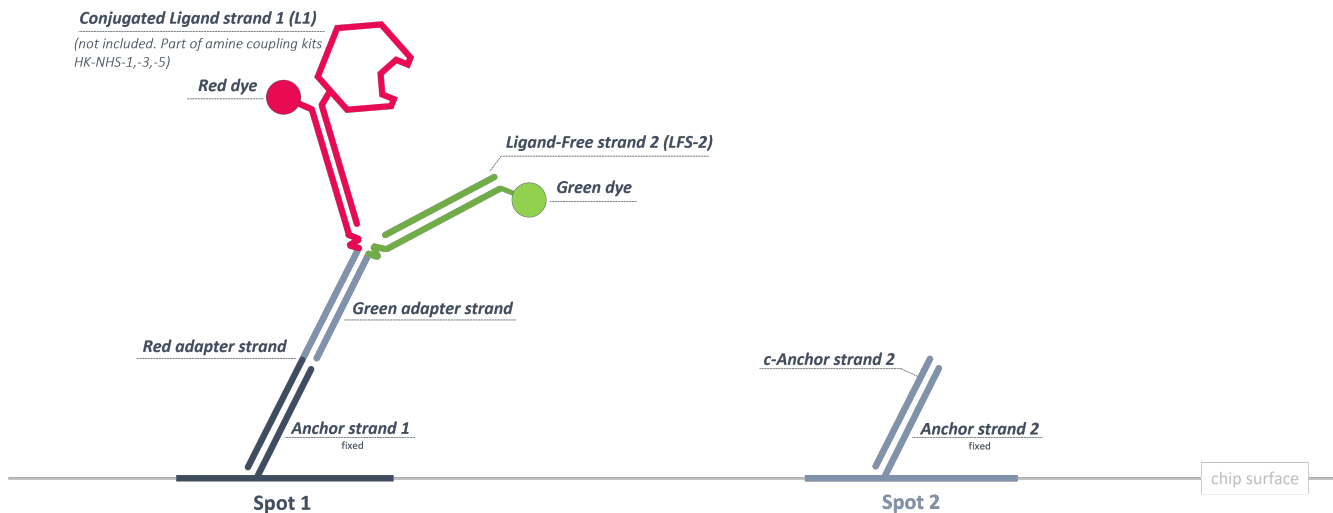
heliX® Adapter Chip Overview

Ternary binding

Conjugated Ligand strand 1 (L1)
(not included. Part of amine coupling kits HK-NHS-1,-3,-5)

Red dye

Conjugated Ligand strand 2 (L2)
(not included. Part of amine coupling kit HK-NHS-4)

Green dye

Green adapter strand

Red adapter strand

c-Anchor strand 2

Anchor strand 1
fixed

Anchor strand 2
fixed

chip surface

Spot 1

Spot 2

Binary binding in green

Ligand-Free strand (LFS-0)

Red dye

Conjugated Ligand strand 2 (L2)
(not included. Part of amine coupling kit HK-NHS-4)

Green dye

Green adapter strand

Red adapter strand

c-Anchor strand 2

Anchor strand 1
fixed

Anchor strand 2
fixed

chip surface

Spot 1

Spot 2

Binary binding in red

Conjugated Ligand strand 1 (L1)
(not included. Part of amine coupling kits HK-NHS-1,-3,-5)

Red dye

Ligand-Free strand 2 (LFS-2)

Green dye

Green adapter strand

Red adapter strand

c-Anchor strand 2

Anchor strand 1
fixed

Anchor strand 2
fixed

chip surface

Spot 1

Spot 2
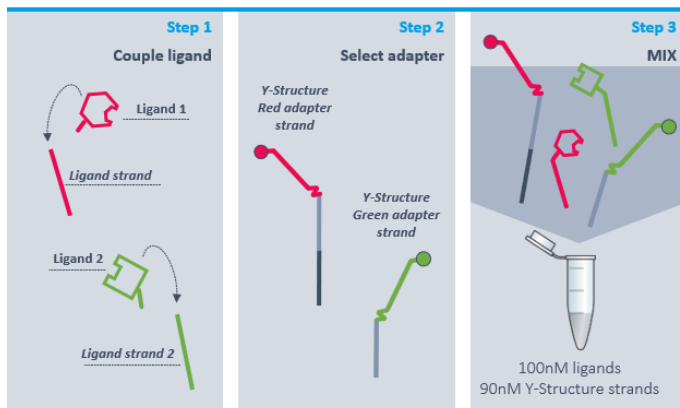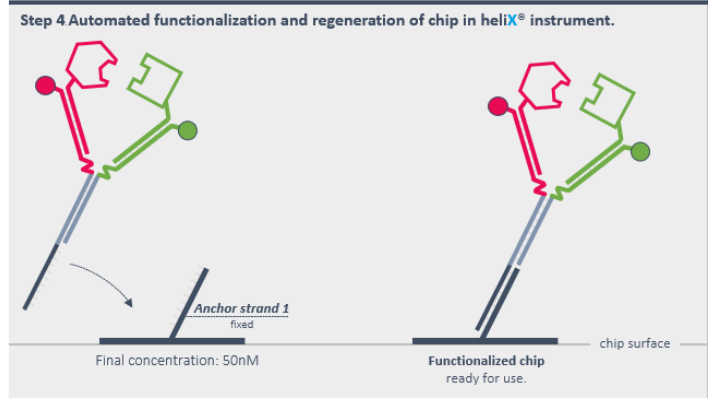
Preparation

Hands-on sample preparation
Couple ligands covalently to ligand strands (e.g., via NHS).

Fully automated workflow

| Step 1 | Step 2 | Step 3 |
| Couple ligand | Select adapter | MIX |

Ligand 1
Ligand strand

Ligand 2
Ligand strand 2

Y-Structure Red adapter strand

Y-Structure Green adapter strand

100nM ligands
90nM Y-Structure strands

Step 4 Automated functionalization and regeneration of chip in heliX® instrument.

Anchor strand 1
fixed

Final concentration: 50nM

Functionalized chip
ready for use.

chip surface

*Incubation suggested: overnight

Choose from **ready-to-use** coupling kits.

Choose from **ready-to-use** adapter strands.

Download the heliX® **MIX&RUN** app for use on your mobile device.

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** 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**. In solution hybridization of **Y-structure** strands:

i. Mix **Y-structure Red Adapter strand with Ra** (400 nM), **Y-structure Green Adapter strand with Ga** (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.

Step 3

Mix solution of step ii) and **cAnchor strand 2** (100 nM) 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 with Ra (400 nM)	*Green Adapter strand with Ga* (400 nM)	Conjugated *Ligand strand* (500 nM)	Conjugated *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

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

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

Dynamic Biosensors, Inc.
300 Trade Center, Suite 1400
Woburn, MA 01801
USA

Order Information   order@dynamic-biosensors.com

Technical Support   support@dynamic-biosensors.com

www.dynamic-biosensors.com

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