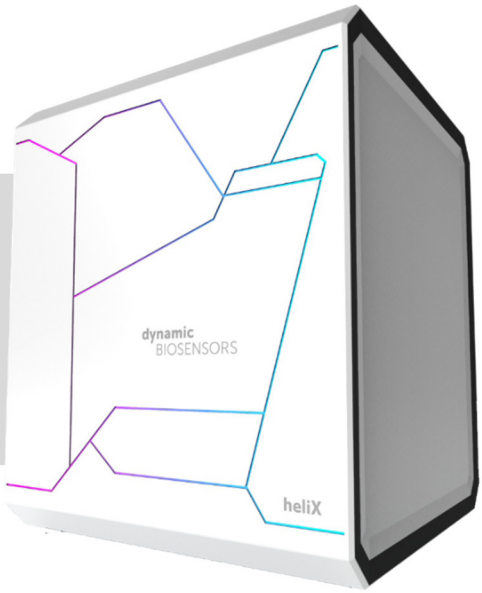
USER MANUAL
DS-6

dynamic BIOSENSORS

heliX® Dye Scouting Kit

with six different dyes (**Ga**, **Gb**, **Gc**, **Ra**, **Rb**, and **Rc**)

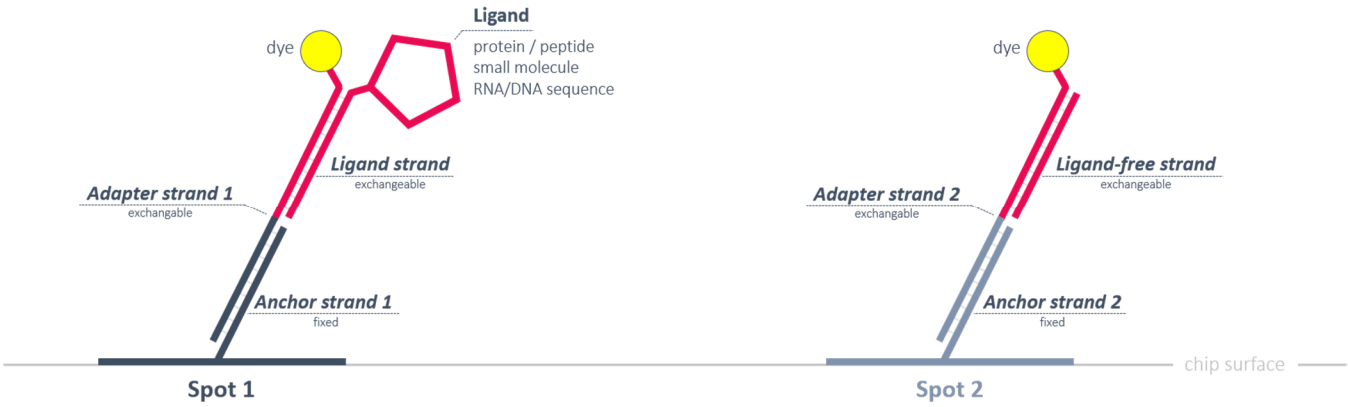


Key Features

- ***Adapter strand 1*** and ***Adapter strand 2*** for functionalization of a **heliX®** Adapter Biochip **Spot 1** and **Spot 2**
- Compatible with all **switch**SENSE® Adapter Biochips
- Includes ***Adapter strands*** for **10 regenerations** for each dye
- Ideal for **MIX&RUN** sample preparation (app available in app stores)
- This kit contains ***Adapter strand 1*** with 6 different dyes and ***Adapter strand 2*** with 6 different dyes prehybridized with free ligand strand. Dye Scouting enables to screen for the most sensitive fluorophore for your application.

heliX® Adapter Biochip Overview

2 spots with 2 different anchor sequences for DNA-encoded addressing



Product Description

Order Number **DS-6**

TABLE 1 | Contents and Storage Information.

Material	Concentration	Amount	Storage
***Adapter strand 1* - Ra (in TE40[1])**	400 nM	100 µL	-20°C
***Adapter strand 1* - Rb (in TE40[1])**	400 nM	100 µL	-20°C
***Adapter strand 1* - Rc (in TE40[1])**	400 nM	100 µL	-20°C
***Adapter strand 1* - Ga (in TE40[1])**	400 nM	100 µL	-20°C
***Adapter strand 1* - Gb (in TE40[1])**	400 nM	100 µL	-20°C
***Adapter strand 1* - Gc (in TE40[1])**	400 nM	100 µL	-20°C
***Adapter strand 2* - Ra - lfs (in TE40[1])**	200 nM	200 µL	-20°C
***Adapter strand 2* - Rb - lfs (in TE40[1])**	200 nM	200 µL	-20°C
***Adapter strand 2* - Rc - lfs (in TE40[1])**	200 nM	200 µL	-20°C
***Adapter strand 2* - Ga - lfs (in TE40[1])**	200 nM	200 µL	-20°C
***Adapter strand 2* - Gb - lfs (in TE40[1])**	200 nM	200 µL	-20°C
***Adapter strand 2* - Gc - lfs (in TE40[1])**	200 nM	200 µL	-20°C

For *in vitro* use only.

To avoid many freeze thaw cycles please aliquot the nanolever.
This product has a limited life time, please see expiry date on label.

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

Preparation | MIX&RUN

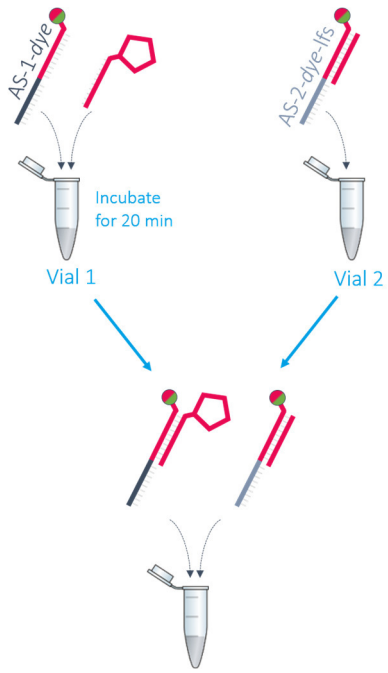
In-solution hybridization of adapter and ligand strands:

1) Mix ***Adapter strand 1*** - dye (400 nM) and conjugated ***Ligand strand*** (500 nM) at a 1:1 ratio[1]

2) Incubate at 25°C at 600 rpm in the dark for 20 minutes to ensure complete hybridization

3) Mix solution of step 2) and ***Adapter strand 2*** - dye - lfs (200 nM) at a 1:1 ratio[1]

Solution is ready to use for biochip functionalization.



Example

Required volume for one functionalization (as suggested in heliOS): **40 µL**

Vial 1		Vial 2
Adapter strand 1 - dye (400 nM)	Conjugated ***Ligand strand*** (500 nM)	***Adapter strand 2*** - dye - lfs (200 nM)
10 µL	10 µL	20 µL

After incubation time, mix vial 1 and vial 2 to obtain 40 µL of ready-to-use DNA solution.

Useful Order Numbers

TABLE 2 | Order Numbers.

Product name	Comment	Order Number
heliX® Adapter Biochip	Chip with 2 detection spots	ADP-48-2-0
heliX® Amine Coupling Kit 1	For five individual conjugation reactions	HK-NHS-1

[1] v/v ratio

[2] If the protein is not stable in PE40 (TE40, HE40), please check buffer compatibility with the switchSENSE® compatibility sheet.

Dye Scouting Information

The two standard fluorescent probes for **switch**SENSE® measurements are the red and green dye **Ra** and **Ga**, respectively. Fluorescence proximity sensing is based on the influence of changes in the local environment on the fluorescence signal of the dye in close proximity. This effect depends on the chemical nature of the dye and the interaction partner. Therefore, a different dye may yield a higher signal response depending on the type of interaction.

This dye scouting kit enables to screen for the most sensitive fluorophore for your application. Three red and three green fluorophores with different chemical properties are available to choose from for your **switch**SENSE® assay. The basic differences of the dyes in terms of net charge and hydrophobicity are depicted below.

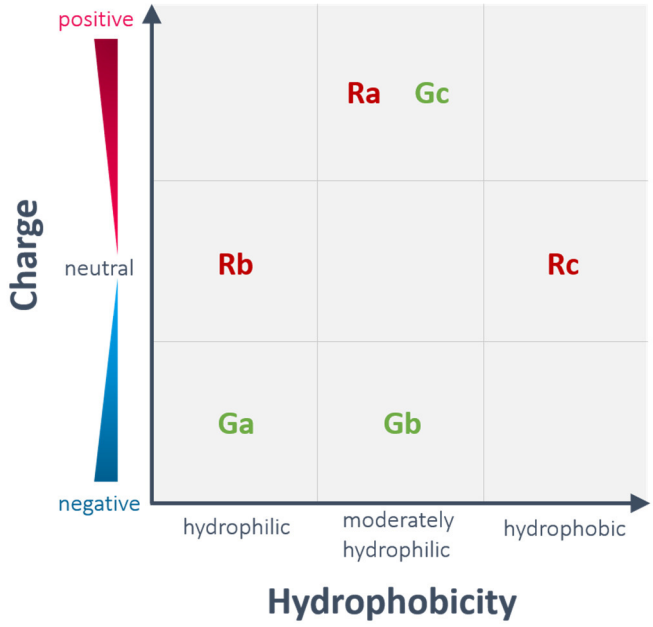


Figure 1: Overview of the **switch**SENSE® dye properties. Six fluorophores - three red dyes (**Ra**, **Rb**, **Rc**) and three green dyes (**Ga**, **Gb**, **Gc**) - are available. Dye Scouting is compatible with the **heliX®** Adapter Biochip. Depending on the interaction partner, different fluorescent probes may obtain different signal responses. Dye scouting allows to quickly screen for maximum signal amplitudes.

Sample Preparation

Perform a MIX&RUN sample preparation for *Adapter strand 1* - dye and *Adapter strand 2* - dye - lfs as described on the previous page and repeat the MIX&RUN sample preparation for all dyes to be tested in the screening. For each ligand immobilization, a volume of 40 µL is required (see example on previous page). Hence, a screening of all six dyes requires 6 vials filled with 40 µL of *Adapter strand 1* - dye and *Adapter strand 2* - dye - lfs (each vial with the respective fluorophore).

Prepare your analyte solution at a sufficient concentration and volume. A high analyte concentration is recommended to ensure saturated association signals (if possible, 10-fold higher than the expected K_D value). The required volume depends on the set association time and is stated in the **heliOS** software (see Figure 3).

Setting up the Dye Scouting Assay in heliOS

Before getting started, open the latest **heliOS** software and connect to your **heliX®** device. Insert an Adapter Biochip into the chip tray, attach your running buffer in the **heliX®** buffer compartment, and prepare a passivation and regeneration solution.

In **heliOS**: Open a new assay tab and add the assay "Dye Scouting", which is a pre-defined method. In this assay the following two steps are performed for each selected dye:

1) **Ligand & Fluorophore immobilization:**
   Spot 1 is functionalized with ***Adapter strand 1***, which carries the fluorophore of interest and is hybridized with conjugated ligand strand.
   Spot 2 is functionalized with ***Adapter strand 2***, which carries the same fluorophore and is hybridized with ligand-free strand (reference).

2) **Analyte binding:**
   Injection of the analyte solution and association to **Spot 1** (real-time reference signal on **Spot 2**) followed by a buffer injection (short dissociation).

The assay set up is shown in Figure 2. Select the tray position of your chip. Define your ligand and analyte name and set the analyte concentration. The association volume corresponds to the amount of analyte required for each measurement run (the dead volume of the vial or well is added in the sample view as shown in Figure 3). The dissociation time is pre-set to 60s but can be modified. A full dissociation is not required for this assay as the aim of this screening is to check for maximal signal amplitudes of the different dyes during association. Hence, a short dissociation time is sufficient to wash away unbound analytes from the surface.

Figure 2 shows the assay setup in **heliOS**. As default setting all six fluorophores are selected. Keep this setting for a full dye scouting run or uncheck any dye, which you want to rule out for your screen. With default parameter settings, the measurement time is around 20 min per dye.

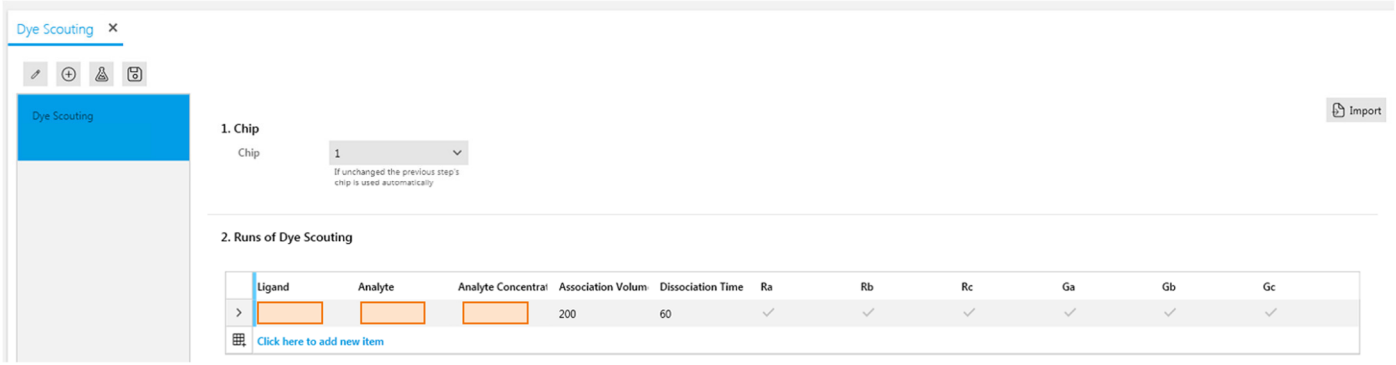


Figure 2: Example of a Dye Scouting assay set up. The assay can be customized. Following parameter settings can be modified: Chip Position, Ligand Name, Analyte Name, Analyte Concentration, Association Volume, Dissociation Time, and Fluorophore Selection.

Once the parameters are defined, save the assay and open the sample tray set up by clicking the respective icon on the top left. Fill the sample tray of your **heliX**® device as indicated in **heliOS**. Figure 3 shows the Sample tray window. After the sample tray is inserted, start the dye scouting assay.

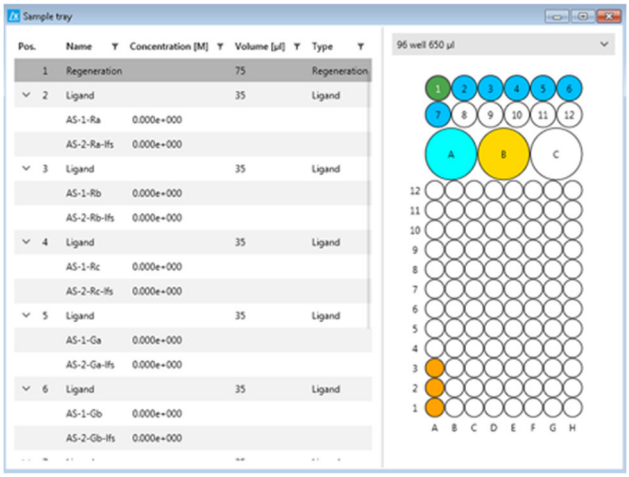


Figure 3: Example of the Sample tray window in **heliOS**. As default setting, all ligands are allocated in vial positions (2 - 12) while analytes are allocated in well positions (A1 - H12). The sample position can be easily changed by drag & drop. Be aware of the different dead volumes when changing from a well to a vial position. The column on the left side shows your sample information, required volume and pre-defined concentration.

Data Analysis in heliOS

Download the data of the Dye Scouting assay from your **heliX**® device and click on "Analyze". Select "Hit Screening" as type of analysis. The software will plot the amplitude signal of analyte association for each tested dye in a bar diagram. Figure 4 shows exemplary data for a full dye scouting run.

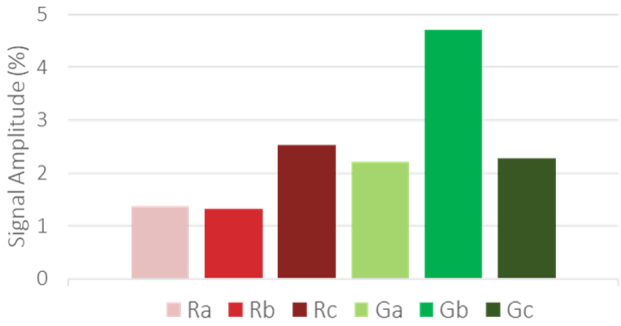


Figure 4: Dye scouting data. Bar diagram shows association amplitudes for each tested dye. For this shown example, **Gb** is the most sensitive dye (5% signal change) and is used for further analysis of this type of interaction.

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dynamic BIOSENSORS MIX&RUN heliX® DNA Calculator

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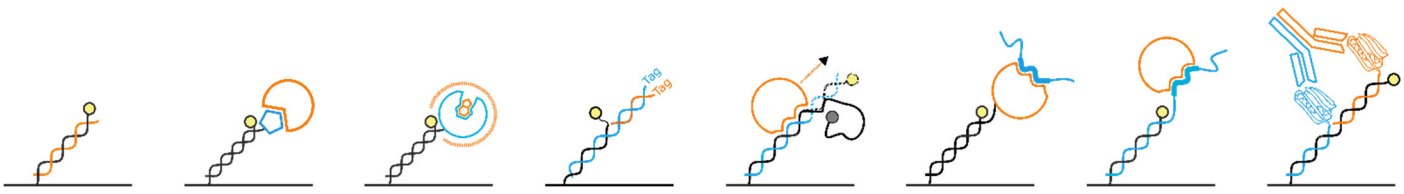

switchSENSE® is a proprietary measurement technology by Dynamic Biosensors GmbH.
Instruments and biochips are engineered and manufactured in Germany.