heliX cyto
User Manual

BRUKER
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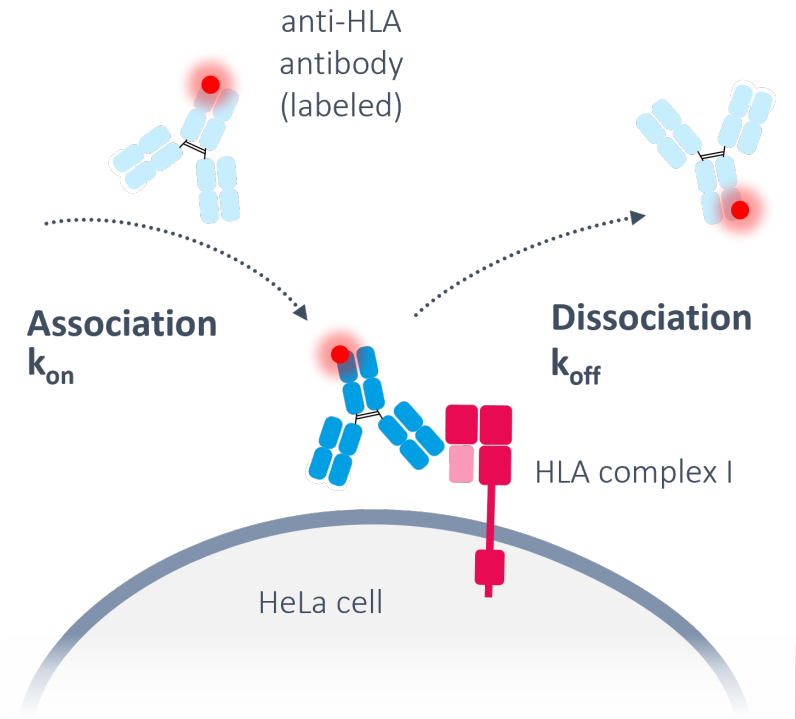
ANTI-HLA ANTIBODY KINETICS TUTORIAL

Manual for conducting an **scIC** experiment for antibody kinetics on cells

Dynamic Biosensors GmbH
CY-DK-HLA-1 v1.0

Key Features

- Capture of fixed eukaryotic cells (HeLa) in cell trap chips in the **heliX**^{cyto}.
- Binding kinetics experiment (k_{on}, k_{off}, K_d) of an antibody interaction on cells: Three concentrations of analyte (anti-HLA antibody) binding to its membrane-bound ligand (HLA beta2-microglobulin) followed by dissociation in buffer flow.
- Assay setup and data analysis with **heliOS** software.



Tutorial Workflow

Set up assay in **heliOS**

Prepare and load samples and **heliX**^{cyto} chip

Run antibody kinetics on **heliX**^{cyto}

Interpret data with **heliOS** automated analysis

Product Description

Order Number: **CY-DK-HLA-1**

Measurement Time: 1:43 h (without priming)

heliOS software version: v2025.1 upwards

The purpose of the Anti-HLA Antibody Kinetics Tutorial kit for **heliX**^{cyto} is to measure binding kinetics of an antibody analyte (anti-HLA) to its matching ligand (beta3-microglobulin of the Human Leukocyte Antigen complex) on a cell surface via single cell Interaction Cytometry (**scIC**). This kit is suitable for the **heliX**^{cyto} device and contains material for 3 kinetics experiments. It includes cell capture sample, analyte solution, and device maintenance solutions.

Use with **heliX**^{cyto} L5 chip.

Table 1. Contents and Storage Information

Material	Cap	Amount	Storage
anti-HLA stock solution (656 nM, red label)	Blue	1 x 70 µL	2-8°C
Cyto test solution	Black	1 x 150 µL	2-8°C
Normalization solution red dye (100 nM)	Orange	1 x 330 µL	2-8°C
Cleaning solution 1 (for **heliX**^{cyto} and chip)	Transparent	3 x 1 mL	15 - 25 °C

The kit contains all required reagents for 3 x 3 concentration kinetics measurements. Upon receipt, store all kit components according to storage temperature in Table 1. Recommended to use **within 2 weeks upon arrival**.

For research use only.

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

Table 2. Additional materials required per run

Material	Cap	Amount	Storage	Comment; *order number*
heliX^{cyto} **L5 chip**	-	1	2-8°C	Measurement chip, reusable; *CY-L5-1*
RB 1	with tube openings	> 150 mL	2-8°C	Running buffer and for analyte dilutions; *BU-RB-10-1*
DI water in large glass vial	**no cap**	10 mL	fresh	-
Small glass vials	black	6	15 - 25 °C	1.3 mL capacity; *AV-015-100N*
Large glass vial	white	1	15 - 25 °C	10 mL capacity; *AV-100-100*

heliX^{cyto} Instrument Preparation

IMPORTANT | *Before starting the tutorial, ensure the* **heliX**^{cyto} *is in a clean state. If needed, run* **Clean & Sleep** *followed by* **Wake Up & Prime** *with fresh solutions and RB 1.*

Place a buffer bottle with enough RB 1 running buffer (at least 150 mL for one experiment) in the buffer compartment of the **heliX**^{cyto}.

IMPORTANT | *In the buffer compartment of the* **heliX**^{cyto} *make sure all 3 tubes are inside the buffer bottle and the ends reach to the bottom of the flask.*

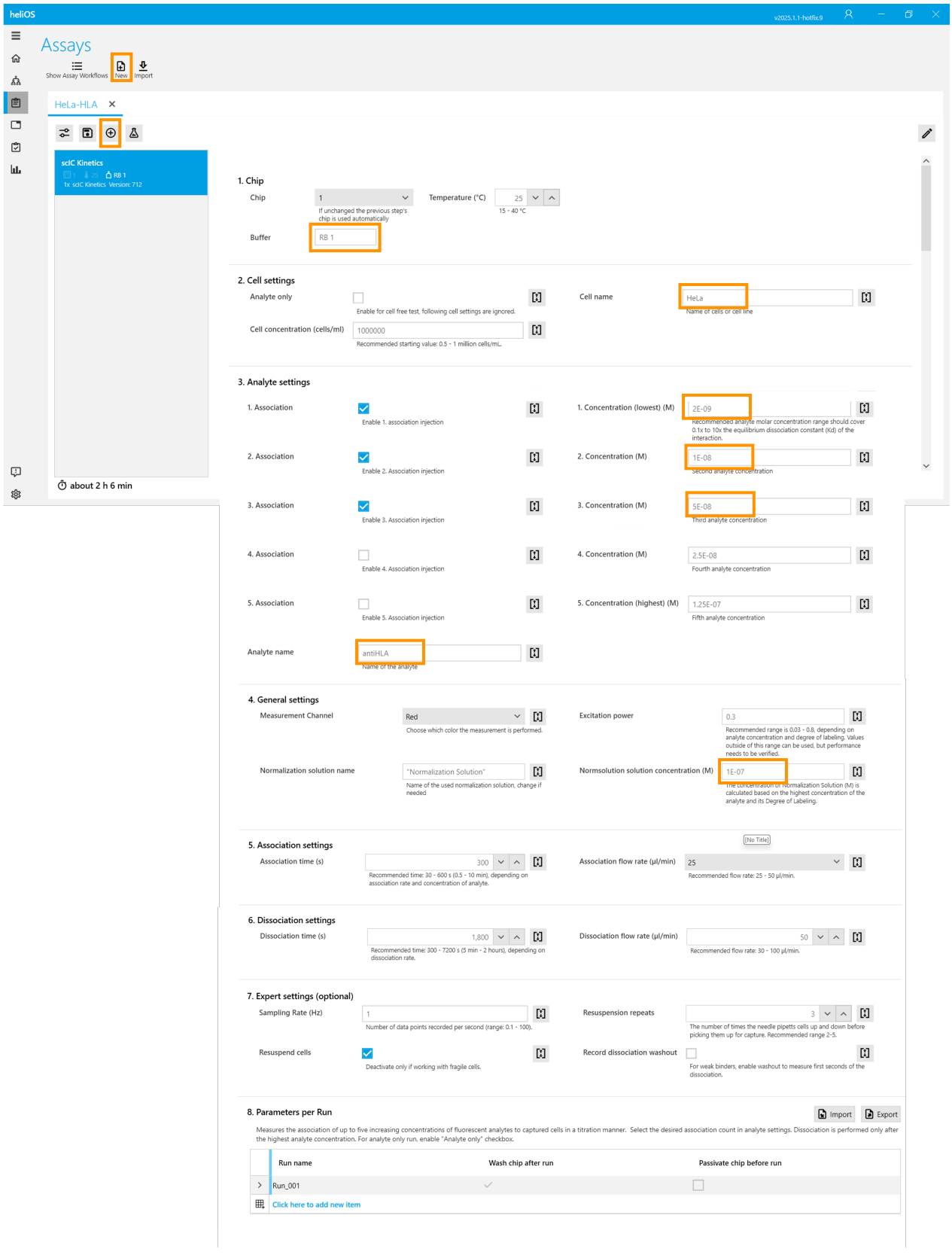
1. Start the latest **heliOS** software version.
2. Go to the **Devices** section of **heliOS** and select the **heliX**® device which you want to use for this assay.
3. Select **Request Control** and wait until the control connection is established. Once this is done, the Request Control will turn into Release Control.
4. Select **Eject Trays**. Remove the chip tray from the compartment and place your **heliX**^{cyto} chip in any of the five chip positions (e.g. position 1). Place a Maintenance Chip for priming and cleaning of the device in position 5. Push the tray back into the compartment until it is fully within the device. Click **Insert Chip Tray** in **heliOS**.
5. Set the **Autosampler temperature** to **15°C** and press the arrow to start the temperature control.

Setting up the Kinetics Assay in heliOS

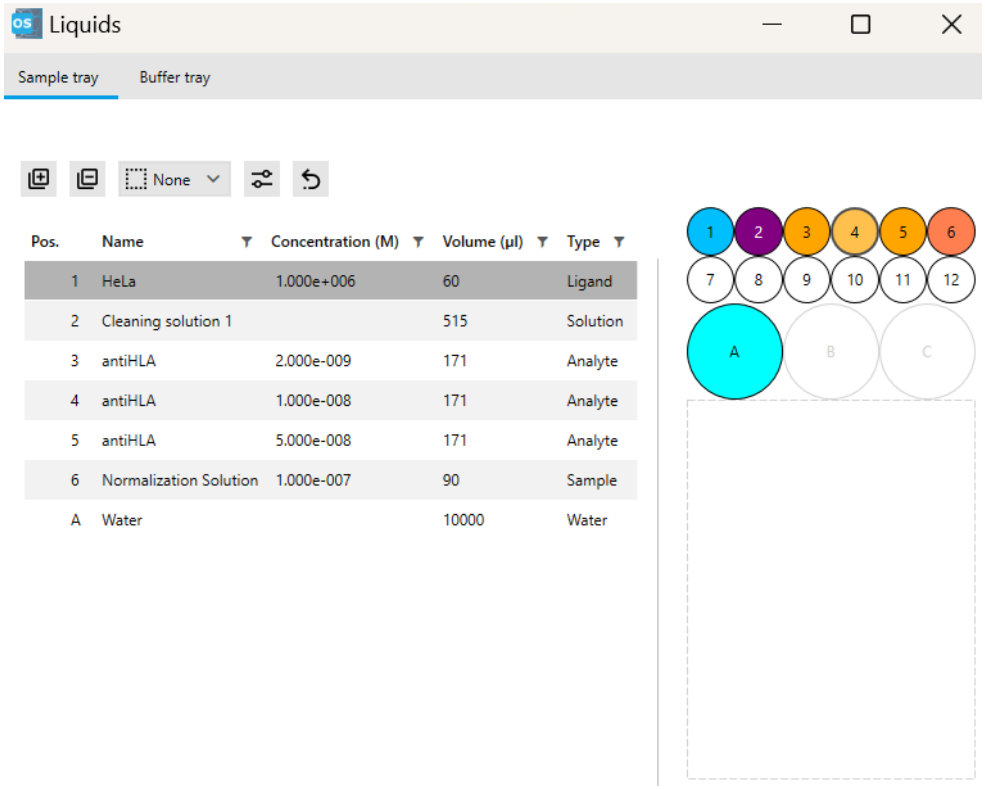
1. Go to the **Assays** section of **heliOS** and click **New** to start creating a new assay. Rename the new assay (e.g. "HeLa-HLA") and Confirm Changes.
2. Add a new **Assay element** to the workflow by clicking the "+" icon.
3. In Custom methods choose **scIC Kinetics** from the assay list (search for "Kinetics" in case of many assays listed or filter by #scIC).
4. Click **Generate and Add Assay**. The assay setup opens with default settings for a full kinetic measurement on cells. Leave all settings at default if not mentioned otherwise in the following:
5. Chip settings: Change the name of the Buffer to **RB 1**.
6. Cell settings: Enter the cell name **HeLa** in the respective field.
7. Analyte settings: Enter the three molar concentrations **2E-9** (lowest), **10E-9**, and **50E-9** (highest) in the first three fields (activated check boxes). Enter **antiHLA** in the respective Analyte name field.
8. General settings: Change the Normalization Solution concentration to **1E-7**.
9. **Save** your assay.

IMPORTANT | *The* **Dissociation time** *may be shortened to* **900 s** *in case of a focus on device testing. Note that the* k_{off} *value may not be determined with confidence in that case.*

IMPORTANT | *Append a* **Cyto System Wash** *directly to the tutorial assay workflow or run it separately after finishing the experiment to clean your* **heliX**^{cyto} *properly.*



10. Open the Sample tray preview (flask symbol) and **deactivate the plate** in the dropdown menu. Then **click on C and B** in the layout to deactivate the large glass vials. The layout should then look like this:



11. **Prepare samples** according to protocol below and place all vials into their indicated positions in the autosampler tray.

Sample preparation per run

(scale up amounts accordingly in case of running replicates)

1. Take samples from the fridge, tap on bench or gently spin down (300 g, 10 seconds) to collect all liquid at the vial bottoms.
2. **Resuspend and transfer** 1 mL (1 aliquot) of provided **CS 1** into a fresh small glass vial for **heliX**^{cyto}.
3. **Resuspend** provided **Normalization solution red dye** and transfer **110 µL** into a fresh small glass vial for **heliX**^{cyto}.
4. Prepare three new small glass vials, label them with the intended analyte concentrations and place indicated amounts of running buffer **RB 1** into the vials:

Table 3. Analyte vial preparation

anti-HLA antibody concentration	RB 1
50 nM	240 µL
10 nM	180 µL
2 nM	180 µL

5. Resuspend **anti-HLA stock** solution with a pipette and transfer **20 µL** into the prepared 240 µL RB 1 for the 50 nM analyte sample. Resuspend well in prepared buffer.
6. Transfer **45 µL** of the 50 nM solution from step 5. with a fresh pipette tip into the vial of 10 nM analyte concentration. Resuspend well in prepared buffer.
7. Transfer **45 µL** of the 10 nM solution from step 6. with a fresh pipette tip into the vial of 2 nM analyte concentration. Resuspend well in prepared buffer.
8. Prepare a new small glass vial with 30 µL of **RB 1** and label it "HeLa". Resuspend **Cyto test solution** gently with a pipette and transfer 30 µL into the prepared buffer. Aspirate and dispense a few times **gently** with pipette.
9. Place sample vials and one large glass vial with DI water (**no cap**) into indicated autosampler tray positions.

IMPORTANT	*Make sure to order the analyte concentrations in the autosampler tray from left to right from* **lowest to highest** *as indicated!*

Starting the Experiment

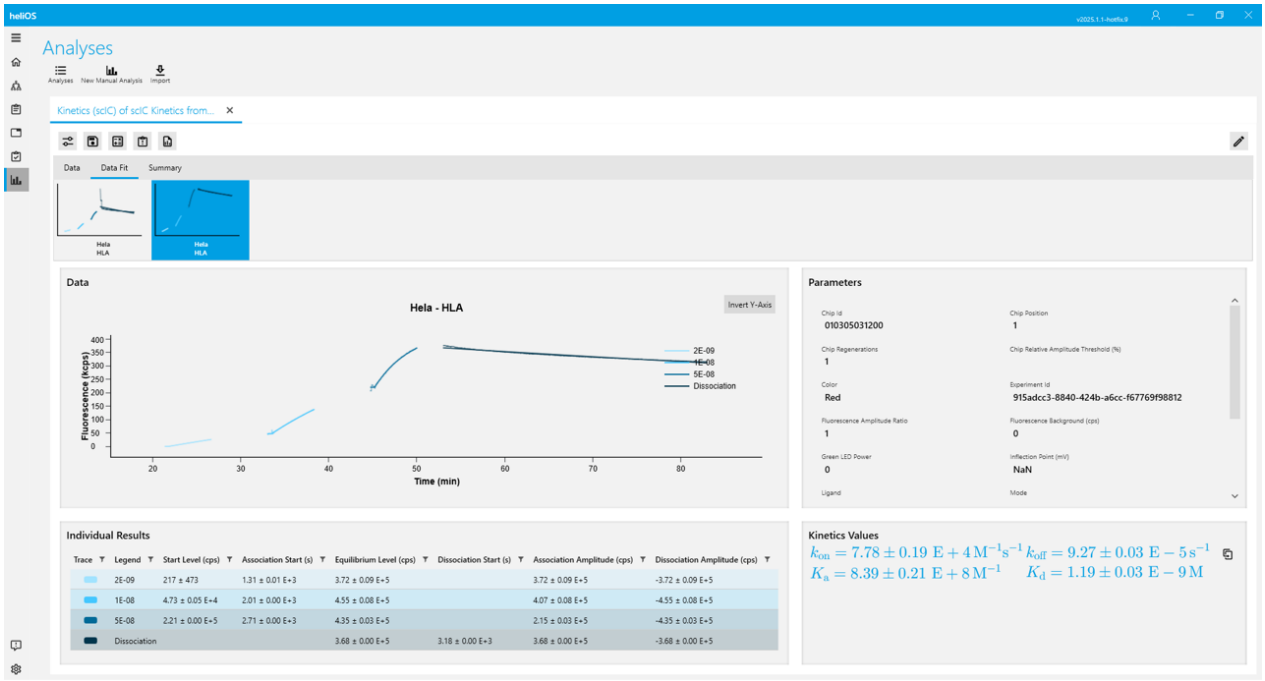
1. After saving the newly generated assay, a **Run** button appears. Click to open the Start Wizard. Select the **heliX®** device which will be used for the measurement. Click **Next**.
2. Push the autosampler tray back into the compartment until it is fully inside the device. Confirm the sample setup by ticking the box **Sample tray is set up as shown** and click **Insert sample tray**. Then click Next.
3. Check that sufficient RB 1 is correctly attached and tick **Buffers are set up correctly**. Click Next.
4. Confirm that the **Chip tray is set up as shown** and click Next.
5. The assay summary shows an overview of the Sample tray, the Chip tray, and the current state of the device. Hit **Start Assay**.

Congratulations, you have started your kinetics experiment on cells!

Data analysis with heliOS

1. Go to **Devices** section in **heliOS** and select your **heliX**^{cyto} device.
2. Open the tab **Experiments** to show all experiments performed on this device. Download the acquired dataset by clicking the **Cloud icon** if it is present. Double-click the dataset to open it.
3. The dataset is opened automatically in the Experiments section of **heliOS**. You can check the timeline of the assay and the images taken throughout. Click the large blue **Analyze** button at the bottom.
4. In the Analysis Wizard: Select the assay block **scIC Kinetics** and click Next. Then select **Kinetics (scIC)** as analysis type and click Next.
5. Leave the Analysis Configuration at **default settings** except for the Fit Model: Keep the default choice of Kinetics - Free End Level but **activate the checkbox** Force Fit End Level to Zero.
6. Click **Analyze** to start the automated analysis. Check your data in the **Data** tab, and find your fit results in the **Data Fit** tab.

Optional: If any minor selection of data to be fit needs to be done, you can do that in the **Manual analysis** (access via right button in series below tab names).



The K_d of the anti-HLA antibody - beta2-microglobulin interaction should be in the single-digit nanomolar range.

Congratulations, you have successfully determined the kinetics of anti-HLA antibody on HeLa cells!

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Instruments and chips are engineered and manufactured in Germany.

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