helix^cyto
User Manual

dynamic
BIOSENSORS

Normalization solution

with red dye **Ra** and green dye **Ga**

Dynamic Biosensors GmbH & Inc.
NOR-0 v1.1

Key Features

- For the normalization of the fluorescent signals on Spot 1 and Spot 2 of a **heliX**^{cyto} chip
- Enables correct real-time referencing of the red or green fluorescent signals during **RT-IC** measurements
- Compatible with all **heliX**^{cyto} chips
- The **Normalization solution-Ra** carries a moderately hydrophilic red dye with a single positive net charge
- The **Normalization solution-Ga** carries a hydrophilic green dye with a single negative net charge

Product Description

Order Number: **NOR-0**

Table 1. Contents and Storage Information

Material	Cap	Concentration	Amount	Storage
Normalization solution-Ra	Orange	10 µM	3x 100 µL	-20 °C
Normalization solution-Ga	Green	10 µM	3x 100 µL	-20 °C

For research use only.

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

Preparation

Choose the normalization solution color (Ga or Ra) corresponding to your analyte label color. Dilute the 10 µM normalization stock solution to a working concentration with running buffer.

The final volume required for the experiment can be found in the **heliOS** sample tray view of the assay.

The concentration of the normalization solution should approximately correspond to the fluorophore concentration in the highest analyte concentration to be measured. This can be calculated using following equation:

$$c_n[M] = c_f[M] = c_a \cdot DOL$$

c_n: Concentration of the normalization solution in the desired color
c_f: Concentration of dye in the labeled analyte solution
c_a: Highest concentration of analyte that should be measured
DOL: Degree of labeling (ratio of dye to analyte)

Diluted solutions can be stored at 2-8°C for up to 7 days.

IMPORTANT: Do not mix Normalization solution-Ra and Normalization solution-Ga together, unless you are setting up a DualColor measurement (parallel read-out of green and red channel).

Application Note

In the RT-IC measurement, the fluorescent signal of the normalization solution should be in a **similar range** as the highest signal coming from bound analyte (raw data).

The absolute fluorescent signal is dependent on normalization solution concentration and the excitation power applied in the measurement. The excitation power has to be selected based on the following parameter:

a. Fluorophore concentration in analyte solution:
   The fluorophore concentration depends on the analyte concentration used in the measurement as well as the degree of labeling of the analyte. For high DOL and high analyte concentrations, lowering the excitation power might be required.

b. Expected binding signal:
   Highly expressed targets on a cell can bind more molecules of labeled analyte. In case of highly overexpressed targets a strong binding signal can be expected. To avoid the shutter closing, lowering the excitation power might be considered.

c. Chip type:
   Different chip types have varying fluorescent background. The bigger the traps and the more traps on the chip, the higher the background signal. Therefore, L5 chips might require lower excitation power than applied to M5 chips.

For a **starting point** of excitation power and norm. solution concentration to be used in an RT-IC experiment, please refer to Table 2.

Table 2. Relation of fluorophore concentration, normalization solution concentration, and excitation power suitable for a **heliX**^{cyto} *M5 chip*

Analyte dye conc. = analyte conc x DOL	Excitation power	Concentration Normalization solution	Dilution Normalization solution
25 nM	0.5	25 nM	1:400
50 nM	0.3	50 nM	1:200
100 nM	0.2	100 nM	1:100
300 nM	0.1	300 nM	1:33
500 nM	0.08	500 nM	1:20
1 µM	0.05	1 µM	1:10
2.5 µM	0.02	2.5 µM	1:4

Note: This table is for your guidance. However, the final signal recorded in the **heliX**^{cyto} *depends on many factors. Thus, some optimization will be required for each system.*

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Instruments and chips are engineered and manufactured in Germany.