



EliGene® LC Spectral Calibration Kit 2

REF 90099-SC2 (for 1 spectral calibration)

Kit components:

1 x 20 µl Calibrator 1
1 x 20 µl Calibrator 2
1 x 20 µl Calibrator 3
1 x 20 µl Calibrator 4
Instruction for Use

Storage and shelf life after first opening:

All components must be transported and stored at -20°C. In the case of thawing the components are stable at 4°C for one day. Unused reagents discard! Protect from light!

Intended use

EliGene® LC Spectral Calibration Kit 2 is intended for spectral calibration of instrument LightCycler® 2.0 for the EliGene® kits.

Protocol for calibration

1. Thaw the four calibrators, Calibrator 1, Calibrator 2, Calibrator 3, Calibrator 4 and shortly spin down.
2. Pipette 20 µl of each calibrator to capillary.
3. Place the capillaries into the carousel on the order: capillary with Calibrator 1 to the first position, capillary with Calibrator 2 to the second position, capillary with Calibrator 3 to the third position and capillary with Calibrator 4 to the fourth position.

Important: For proper calibration the standards must be in this order!!

4. Select protocol New Experiment and you will be presented with a default Run Module. Create a single program
5. (1 cycle) with the following segments:
Segment 1: 95°C for 0 seconds at 20°C/second Acquisition Mode: None
Segment 2: 40°C for 30 seconds at 20°C/second Acquisition Mode: None
Segment 3: 95°C for 0 seconds at 0.1°C/second Acquisition Mode: Continuous
6. Choose Color Compensation from the Analysis Mode drop-down menu.
7. Set the seek temp to 45°C. Set the Max. Seek Pos. to "4" indicating the number of capillaries you have loaded into the instrument, and then switch to the Sample Module.
8. Give the samples in each position appropriate names. Position 1 = Calibrator 1, Position 2 = Calibrator 2, Position 3 = Calibrator 3, Position 4 = Calibrator 4.
9. After naming the samples, select the Analysis Type drop-down menu and choose Color Compensation.



10. You will now need to select the dominant channel (filter) that should be used to measure the fluorescent reporter for each reaction. For your capillary containing Calibrator 1 position 1, the dominant channel should be Water. For the Calibrator 2, the dominant channel should be 530. For Calibrator 3, the dominant channel should be 705. For Calibrator 4, the dominant channel should be 555.
11. **Important: this decision will determine the channels used to detect this combination of fluorescent reporters in your future experiments.**
12. After choosing the dominant channels for each of your samples, switch back to the Run Module and click Start Run. You will be prompted to save the experimental file with an appropriate name and the run will begin.
13. Before beginning the thermal cycle, the instrument will “seek” the position of the capillaries as a part of optimization of the optics. If the instrument fails to locate one or more of the samples, it may be necessary to abort the run so that it may be re-programmed, this time with a higher seek temperature.
14. Once the run is completed, click on the Analysis panel and select Color Compensation beneath the other Methods category. You will be presented with a Color Compensation analysis module where you can view the fluorescence of each reporter with respect to temperature.
15. At the top of this Color Compensation Module, select Save CC Object. You will be prompted to name the Color Compensation file, and the location where it will be saved (usually the CCC directory).
16. This Color Compensation is now available to apply to your future experiments with EliGene LC kits.

Applying of the calibration

To apply Color Compensation to your results, Select Color Compensation from the Color Compensation dropdown menu of any analysis module. Cross-talk of fluorescent reporters will be subtracted out of their non-dominant channels.

This kit is intended for in vitro use only.

Warnings and general precautions

- Handle and dispose of all biological samples as if they were capable of transmitting infective agents. Avoid direct contact with the biological samples. Avoid splashing or spraying. The materials that come into contact with biological samples must be treated with 3% sodium hypochlorite for at least 30 minutes or autoclaved at 121 °C for one hour before disposal.
- Handle and dispose of all reagents and all assay materials as if they were capable of transmitting infective agents. Avoid direct contact with the reagents. Avoid splashing or spraying. Waste must be treated and disposed of in compliance with the appropriate safety standards. Disposable combustible materials must be incinerated. Liquid waste containing acids or bases must be neutralized before disposal.
- Wear suitable protective clothing and gloves and protect eyes/face.
- Never pipette solutions by mouth.
- Do not eat, drink, smoke or apply cosmetic products in the work areas.



- Wash hands carefully after handling samples and reagents.
- Dispose of leftover reagents and waste in compliance with regulations in force.
- Read all the instructions provided with the kit before running the assay.
- Follow the instructions provided with the kit while running the assay.
- Do not use the kit after the expiry date.
- Only use the reagents provided in the kit and those recommended by the manufacturer.
- Do not mix reagents from different batches.
- Do not use reagents from other manufacturer's kit.

Warnings and precautions for molecular biology

- Molecular biology procedures, such as extraction, reverse transcription, amplification and detection of nucleic acids, require qualified staff to prevent the risk of erroneous results, especially due to degradation of the nucleic acids contained in the samples or due to sample contamination by amplification products.
- It is necessary to have separate areas for the extraction/preparation of amplification reactions and for the amplification/detection of amplification products. Never introduce an amplification product in the area designed for extraction/preparation of amplification reactions.
- It is necessary to have lab coats, gloves and tools which are exclusively employed in the extraction/preparation of amplification reactions and for the amplification/detection of amplification products. Never transfer lab coats, gloves or tools from the area designed for the amplification/detection of amplification products to the area designed for the extraction/preparation of the amplification reactions.
- The samples must be exclusively employed for this type of analysis. Samples must be handled under a laminar safety box. Tubes containing different samples must be never opened at the same time. Pipettes used to handle samples must be exclusively employed for this specific purpose. The pipettes must be of the positive displacement type or be used with aerosol filter tips. The tips employed must be sterile, free from DNases and RNases, free from DNA and RNA.
- Reagents must be handled under PCR box or laminar flow box. The reagents required for amplification must be prepared in such a way that they can be used in a single session. The pipettes employed to handle the reagents must be used exclusively for this purpose. The pipettes must be of the positive displacement type or be used with aerosol filter tips. The tips employed must be sterile, free from DNases and RNases, free from DNA and RNA.
- Amplification products must be handled in such way as to reduce dispersion into the environment as much as possible, in order to avoid the possibility of contamination. Pipettes used to handle amplification products must be employed exclusively for this specific purpose.



Warnings and precautions specific to components of the kit

- The plate containing calibrators (Calibrator 1, Calibrator 2, Calibrator 3 and Calibrator 4) are disposable and therefore must be used once only in the preparation of the reaction mixture.
- This plate carries the following safety warnings (P):

P280 Wear protective gloves/protective clothing/eye protection/face protection.

P281 Use personal protective equipment as required.

Symbols



Catalog number



Upper limit of temperature



Batch code



Use by (last day of month)



in vitro diagnostic medical device



Fulfilling the requirements of European Directive 98\79\EC for *in vitro* diagnostic medical device.



Contains sufficient for "N" tests



Attention, consult instructions for use



Manufacturer

Manufacturer

ELISABETH PHARMACON Ltd.

Rokycanova 4437/5, 615 00 Brno, Czech Republic Tel.: +420 542 213 851, +420 542 213 827

E-mail: info@elisabeth.cz