

Instructions for Use

BAG RT-PCR Universal Custom Dye Calibration Kit

RUO

EN

REF 728260 RT CC Universal Applied Biosystems®

For use on the Applied Biosystems® RT-Instruments

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1. Application

The universal custom dye calibration kit for Applied Biosystems™ real-time PCR instruments is used to run assays designed with custom dyes which are not manufactured by Thermo Fisher Scientific. The universal custom dye calibration kit is to be used in combination with BAG Diagnostics real-time PCR kits: FastQ®, ERY Q® and HISTO TYPE Rainbow. Once the custom dye calibration has been performed the new dyes get added to the Dye Library of the real-time PCR - cyclers and can be used for running new experiments.

For research use only

2. Product description and principle

Due to the overlap of the emission spectra of organic dyes, crosstalk emission between detector channels can occur. This phenomenon is described as the overspill of one dye into the next detector channel which may result in the misinterpretation of the data. To correct for cross-talk emission between detector channels, dye calibration is needed for analyzing the data. The software uses calibration data to characterize and distinguish the individual contribution of each dye in the total fluorescence signals collected by the instrument. The software extracts a spectral profile for each dye standard, then produces a set of spectral profiles plotted as fluorescence versus filter. The pass criteria for a dye calibration is that the dye spectra peak within the same filter as their group [1].

3. Kit contents for custom dye calibration

Components	Description	Concentration	Storage conditions
ORANGE 560 custom dye standard	1 orange cap tube 100 µl	10 µM	≤ -20°C
RED 610 custom dye standard	1 red cap tube 100 µl	10 µM	
QUASAR 670 custom dye standard	1 purple cap tube 100 µl	10 µM	

4. Additionally required reagents and devices

- Variable pipettes (0,5 – 1000 µl) and pipette tips
- Application spatula for PCR foil
- Molecular grade DNase free water.
- Centrifuge for PCR plates
- PCR Plate (see cycler specific requirements)
- PCR Foil (see cycler specific requirements)

5. Storage and stability

The kits are shipped on blue ice. Upon receipt store all reagents in temperature monitored devices at ≤ -20°C. The manufacturer date is indicated on the label of each reagent. Please avoid multiple freeze-thaw cycles.

6. Test procedure

6.1 Safety conditions and special remarks

Molecular genetic techniques are particularly sensitive and should be performed by well trained personnel experienced in molecular genetic techniques.

Special safety conditions must be observed in order to avoid contamination and thus false reactions:

- Wear gloves during work (powder-free, if possible).
- Use new tips with each pipetting step (with integrated filter).
- Use separate working areas for pre-amplification (DNA isolation and PCR set up) and post-amplification (detection). Preferably, use two separate rooms.
- Use devices and other materials only at the respective places and do not exchange them.

6.2 Custom dye calibration

Due to different Applied Biosystems™ real-time PCR instruments and systems it is not possible to list all workflows for each cycler system. For more information about the custom dye calibration workflow for your instrument or system, please contact your local Applied Biosystems™ technical support or use the latest Custom Dye Calibration of Applied Biosystems™ Real-Time PCR Instruments User Bulletin. For further or additional information about custom dye calibration please contact the technical support of BAG Diagnostics (phone: +49 (0)6404 925125, email: info@bag-diagnostics.com) or your local sales representative.

7. Explanation of symbols used on the labels

	Storage temperature / Lower limit of temperature
	Use by
	Consult instructions for use
	Manufacturing Date
	Manufacturer
eIFU VXX/XXXX	Electronic instructions for use Version of the actual instruction for use
RUO	For research use only
LOT	Batch code
CAL O560	Orange 560 custom dye standard
CAL R610	Red 610 custom dye standard
CAL Q670	Quasar 670 custom dye standard
REF	Catalogue number

8. References

[1] Custom Dye Calibration of Applied Biosystems™ Real-Time PCR Instruments User Bulletin