Microsynth

Multi-Capillary Microsynth Matrix Standard Kit (Mic-G5)

Calibrate Your Sequencer for Optimal Use of Microsynth's Cost-Effective Dyes

Dear Customer
When calibrating your ABI sequencer for optimal utilization of Microsynth's cost-effective dye alternatives instead of the patent-protected dyes VIC, NED, PET and LIZ, please consider the following advices:

General Remarks

A proper spectral calibration file is the basis of multicolor capillary electrophoresis using the ABI Genetic Analyzers. The Microsynth Matrix Standard (Mic-G5-Matrix-Std) contains a mixture of five DNA fragments labeled with FAM, ATTO 532, ATTO 550, ATTO 565 and Dyomics 630 respectively (see table below). It is recommended for the use with ABI 3100, 3130, 3500, 3700 and 3730 series

Matrix Standard Type/Channel	Blue	Green	Yellow	Red	Orange
Matrix Standard Kit (Mic-G5) from Microsynth	FAM	ATTO 532	ATTO 550	ATTO 565	Dyomics 630
DS-33 Matrix Standard Kit (Dye Set G5) from ABI	FAM	VIC	NED	PET	LIZ

There are some instrument to instrument variation in the sensitivity of detection. The optimal range of the calibration signal's peak heights is 1'000 – 4'000 rfu. If the calibration applying the following method leads to much to high peaks, it may be necessary to perform a second calibration run applying optimized parameters (e. g. shortened injection time, reduced injection voltage or just a higher dilution of the matrix standard than described here).

Storage Conditions

The fragments in the matrix standard are light sensitive and must be stored in the dark. Store the Mic-G5-Matrix-Std tube in the freezer up to its use. Do not store reagents in the freezer door, where the temperature can fluctuate.

An arranged calibration plate can be stored for reusing in the freezer up to a year.

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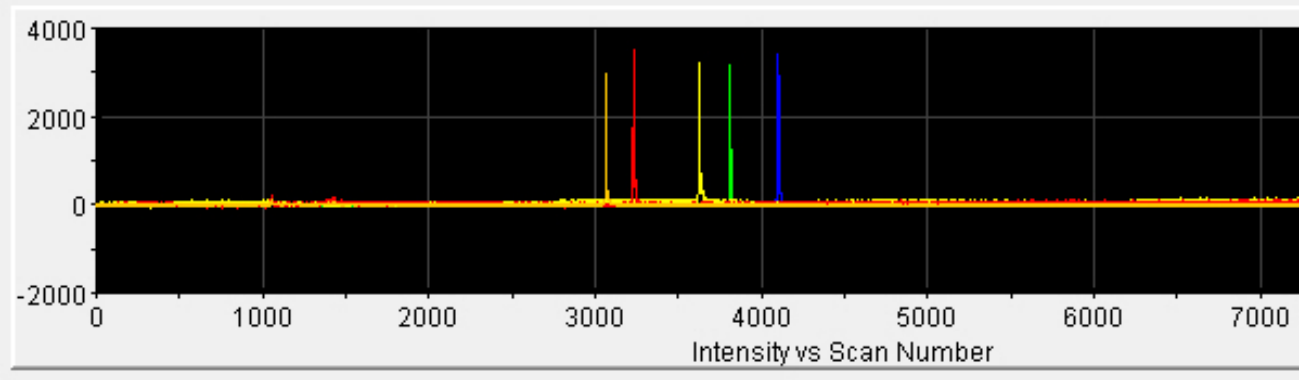
Method

1. Thaw and thoroughly mix the contents of the Microsynth Matrix Standard tube (Mic-G5- Matrix-Std). Centrifuge the tube briefly.
2. Add 1'000 µl Hi-Di-Formamide (500 µl Hi-Di-Formamide for tubes marked with: 48) to the tube. Mix the content by vortexing the tube for 5 – 10 seconds. Centrifuge the tube briefly.
3. Dispense 10 µl of the arranged mix per well in a 96-well plate and briefly centrifuge the plate.
4. Denature the calibration plate just prior to loading the instrument: Heat the samples at 95°C for 3 minutes, then chill down immediately on crushed ice or in an ice water bath for 3 minutes.
5. Perform a spectral calibration run according to your sequencer's manual. Choose the instrument protocol for 5 Dyes (G5) belonging to the MtxStd-run module. (Check the parameters predefined in the Instrument Protocol: increase the range of Matrix Condition Number Bounds to 4.0 – 14.5.).
6. After the calibration run check the calibration quality in the Event Log Window. If a single capillary did not pass the quality control only, the software uses the calibration values of the neighbor's capillary.
7. If (most of) the signal height of the matrix standard fragments is saturated, repeat the calibration run with diluted calibration plate, shortened injection time or reduced injection voltage.
8. Rename the quality passed matrix for future capillary electrophoresis applications.

Result

Example electropherogram of a typical calibration using the Microsynth Matrix Standard Mic-G5.



Need More Information?
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