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Application Guide

RainDrop® Digital PCR System

Utilizing Universal Exogenous Internal Positive Controls to Distinguish True Target Negatives from False Negatives

Purpose and Scope

Real-time PCR assays are prone to inhibition by various substances found in many samples. Eurogentec's Universal Exogenous FAM Internal Positive Control (IPC) kit (PN RT-IPCF-B01) has been optimized for the RainDrop Digital PCR System, enabling users to distinguish true target negatives from false negatives due to PCR inhibition, incorrect pipetting or cycling parameters.

The kit can be utilized as an assay-based internal positive control and as a validation tool for the RainDrop® Digital PCR System.

Image 1 : RainDance's RainDrop Digital PCR System.

Assay-based Internal Positive Control

The kit can be used to evaluate the quality of samples and assays by spiking the control DNA and assay into a reaction, then verifying that the control cluster is present. In Figure 1, the FAM IPC kit was added to a SNP assay, with a VIC-labeled WT probe and a FAM-labeled mutant probe. Note the IPC cluster located between the non-amplified cluster and the FAM-mutant cluster, on the X-Axis.

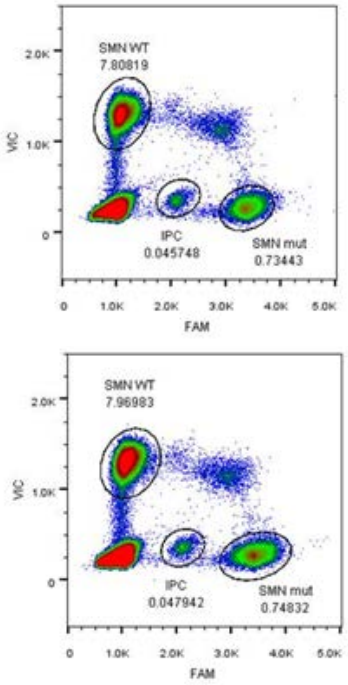

Figure 1: FAM IPC Kit added to existing duplex assay.

RainDrop System Validation Tool

The assay can also be run as a single-plex to verify instrument performance. Figure 2 shows the expected cluster placement. Please note that single-plex assays are not spectrally-compensated.

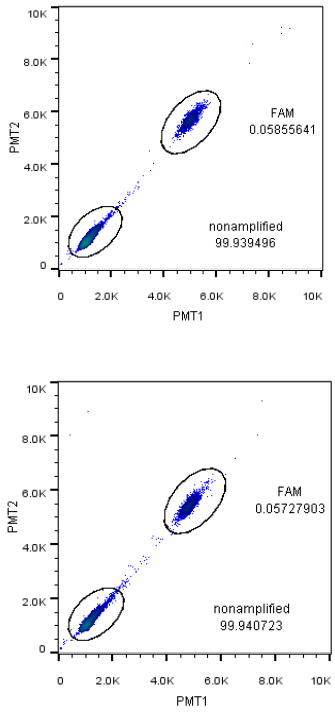

Figure 2: FAM IPC kit run alone as a single-plex.

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Protocol for Running FAM IPC Kit

Please reference the Eurogentec (EGT) Universal Exogenous Internal FAM Positive Control (PN RT-IPCF-B01) Technical Data Sheet for further information.

The kit contains 2 tubes:

1. 10X EGT IPC mix, 550 µl, which contains the IPC primers and FAM probe
2. 50X EGT IPC DNA, 110 µl, containing the template DNA

For long term storage, the kit should be kept in the dark at -20˚C. The kit is stable at 4˚C for up to one month. Avoid freeze thaw cycles, and protect the IPC mix from light.

Reaction Mix

Reagent	25 µl reaction	50 µl reaction
2x Master Mix	12.5 µl	25.0 µl
10X IPC mix	2.5 µl	5.0 µl
50X IPC DNA	0.5 µl	1.0 µl
25X droplet stabilizer	1.0 µl	2.0 µl
Total	16.5 µl	33.0 µl
Remaining	8.5 µl	17.0 µl

The remaining volume contains any additional primers, probes, template DNA (if added) and water.

Thermal Cycling Conditions

- If the FAM IPC kit is being spiked into an existing assay, run the reaction using the normal thermal cycling conditions for that assay.
- If running the kit in a single-plex reaction, use the following thermal cycling conditions:

Step	Temperature	Time	# Cycles
Enzyme Activation	95°C	10 min	1
Denaturation	95°C*	15 sec	45
Annealing	51°C*	15 sec	
Extension	60°C	60 sec	
Enzyme Deactivation	98°C	10 min	1
Hold	4°C	Infinite**	

**Hold at 12˚C for at least 15 min.