Feedback from a Customer

Product: **FastGene® 0.2 ml PCR tubes with flat caps (FG-021F)**

Manufacturer: **NIPPON Genetics Co., Ltd**

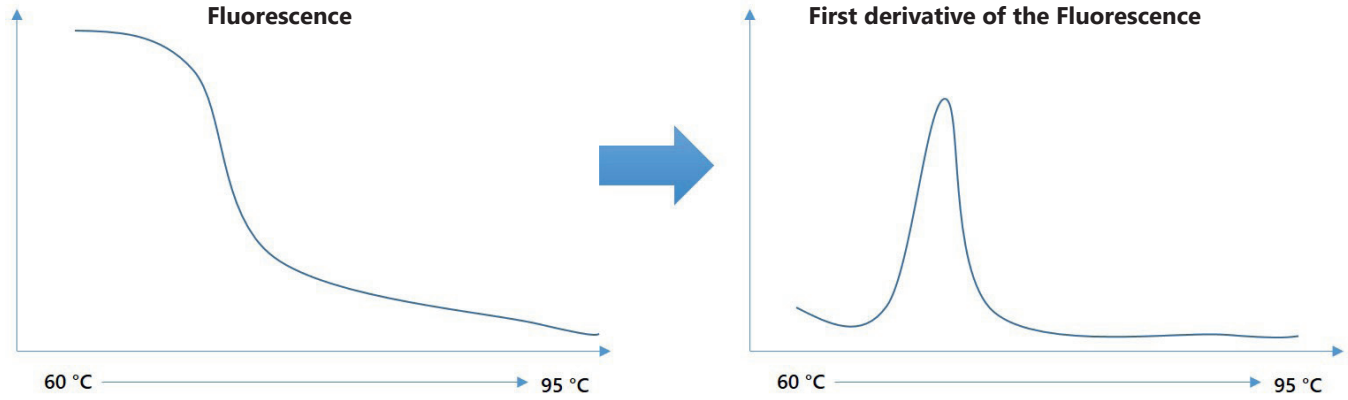
Application: **PCR-tubes (FG-021F) in High-Resolution Melt (HRM) analysis on a Rotor-Gene® Q**

The here presented data was provided by the courtesy of Dr. Birgit Klinkert, ARDEYPHARM GmbH, Herdecke, Germany

Background

The quantification and detection of genes can be performed using quantitative real-time PCR. The detection of nucleic acid is performed using a intercalating dye called SYBR®. The fluorescence of this dye increase when bound to DNA. Therefore the intensity of the fluorescent signal is directly dependent on the concentration of double stranded DNA.

Additionally to the quantification of DNA, an intercalating dye can also be used to analyse the fragment. Depending on the sequence, different amount of thermal energy is needed to denature a double stranded DNA fragment into a single stranded. Hence, measuring the fluorescence signal while melting the double stranded DNA to single stranded DNA causes a reduction of the fluorescence signal.



Methods

- **PCR-Tubes**

  1) FastGene® 0.2 ml PCR tubes with flat caps (Cat.No. FG-021F)
  2) Original 0.2 ml Rotor-Gene® tubes (Cat.No. 981005)

- **qPCR Instrument** QIAGEN® Rotor-Gene® Q Mdx 5plex

- **qPCR Cycling Conditions**

qPCR

95 °C 5 min

94 °C 15 sec
53 °C 20 sec
72 °C 45 sec
] x 35 cycles

HRM-Analysis

Starting temperature 60 °C
Final temperature 90 °C
] 0.5 °C increments

- **qPCR Volumes** 25 µl reaction volume

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Results

The fluorescence curve of two different primer pairs were analysed over 30 cycles in the original PCR tube (dark color) or in FG-021F (light color) and are shown in the figure 2. The C_T-value detected by the qPCR instrument are summarized in Table 1. The results of the melting curve analysis are shown in table 2.

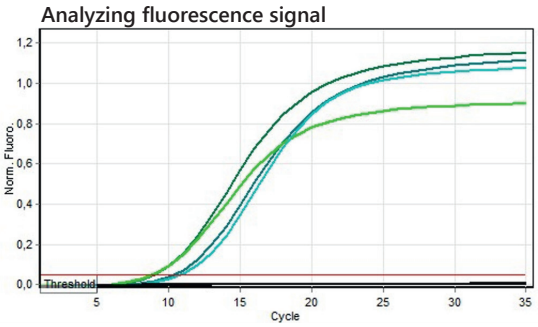

Table 1: Measured C_T-values

Primer pairs	C_T QIAGEN® tube	C_T FastGene® tube	NTC
Gene 1	9.01	8.88	n.d | n.d
Gene 2	10.38	10.91	n.d | n.d

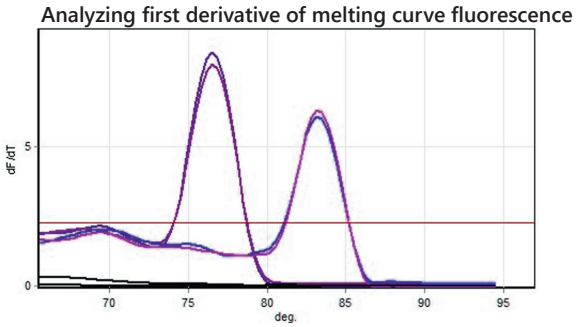

Table 2: Measured temperature peak

Primer pairs	Peak QIAGEN®	Peak FastGene®	NTC
Gene 1	76.5 °C	76.5 °C	n.d | n.d
Gene 2	83.2 °C	83.3 °C	n.d | n.d

Fig. 2: Fluorescence mesaurement of four different probes in a multiplex reaction.

<Conclusion>

The FastGene® 0.2 ml PCR tubes does not interfere in the detection of the fluorescence of SYBR® Mix. The measured C_T-values were comparable or better. The analysis of the first derivative of the fluorescence during high resolution showed no difference between the two different tubes.

<Customer's comments>

The lid of the FastGene® 0.2 ml PCR tubes are different from the original. Nonetheless, the lock mechanism of the Rotor-Gene® Q Mdx worked perfectly with them. The fluorescence of the probes in the reaction are measured at the tip of the tubes. We can recommend to replace the original tubes for the here tested fluorescent probes without any restriction.

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