QIAGEN Supplementary Protocol:

LightCycler® 1.x Software Setup for the QuantiFast™ SYBR® Green RT-PCR Kit

This protocol shows the necessary parameters that need to be entered into the LightCycler 1.x software (version 3.5) when using the QuantiFast SYBR Green RT-PCR Kit.

IMPORTANT: Please read the *QuantiFast SYBR Green RT-PCR Handbook*, paying careful attention to the safety information, before beginning this procedure. The QuantiFast SYBR Green RT-PCR Kit is intended for research use. No claim or representation is intended to provide information for the diagnosis, prevention, or treatment of a disease.

Procedure

1. Launch the software and set up the cycle program data as described in the next few steps.

2. Select "None" and set up the parameters for the reverse-transcription step as shown below.

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3. **Select "None" and set up the parameters for the initial activation step as shown below.**

4. **Select "Quantification" and set up the parameters for PCR cycling as shown below. Be sure to select "SINGLE" for "Acquisition Mode" at the 60°C step.**

5. Select "Melting Curves" and set up the parameters for melting curve analysis as shown below. Be sure to select "CONT" for "Acquisition Mode" at the 95°C step.

6. Select "None" and set up the parameters for cooling as shown below.

7. Load your PCR capillaries and start the program.

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