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Invitrogen SuperScript™ III First-Strand Synthesis SuperMix for qRT-PCR
Cat. No. 11752-050 Size: 50 reactions

Protocol for First-Strand cDNA Synthesis

The following protocol has been optimized for generating first-strand cDNA for use in two-step qRT-PCR. Note that an incubation temperature of 50°C for 30 minutes is recommended as a general starting point. Higher temperatures (up to 60°C) may be used for difficult templates.

1. Combine the following kit components in a tube on ice. For multiple reactions, a master mix without RNA may be prepared:
2X RT Reaction Mix 10 µl
RT Enzyme Mix 2 µl
RNA (up to 1 µg) x µl
DEPC-treated water to 20 µl

2. Gently mix tube contents and incubate at 25°C for 10 minutes.

3. Incubate tube at 50°C for 30 minutes.

4. Terminate the reaction at 85°C at 5 minutes, and then chill on ice.

5. Add 1 µl (2 U) of *E. coli* RNase H and incubate at 37°C for 20 minutes.

6. Use diluted or undiluted cDNA in qPCR, or store at –20°C until use.

Note: Up to 10% of the qPCR reaction volume may be undiluted cDNA (*e.g.,* for a 50-µl qPCR, use up to 5 µl of undiluted cDNA from Step 6 above).

Note: 1/20 of the final product will be used for RT-PCR per reaction. To avoid the pipetting error with such a low volume pipetting, dilute the final to 5x (then use 5ul per reaction).