ReadiScript™ RT Reverse Transcription Kit

Catalog number: 60100
Unit size: 50 Reactions

Component	Storage	Amount (Cat No. 60100)
Component A: ReadiScript™ RT Enzyme	Freeze (< -15 °C)	1 vial (50 µL)
Component B: ReadiScript™ RT Reaction Mix (5X)	Freeze (< -15 °C)	1 vial (250 µL)

OVERVIEW

The ReadiScript™ RT Reverse Transcription kit is a convenient tool for cDNA synthesis used in gene expression analysis with qPCR. The two-tube kit format helps to set up the reaction faster and is optimized to yield cDNA over a broad dynamic range. The 5X supermix contains a unique blend of oligo(dT) and random primer mix to prevent the 5′ and 3′ bias of the target genes. The reaction mix contains a potent blend of RNase inhibitors to protect RNA during the setup and reverse transcription process. The resulting cDNA product is compatible with all qPCR chemistries or conventional end-point RT-PCR.

KEY PARAMETERS

Thermal Cycler

SAMPLE EXPERIMENTAL PROTOCOL

Allow the ReadiScript™ RT Reaction Mix (5X) to thaw on ice. Before using, vortex reaction mix thoroughly.

The following protocol can be used as a guideline.

1. Prepare the cDNA synthesis reaction by adding the following components to a microcentrifuge tube in the order they appear below:

   Table 1. Reagents composition per well for each reaction.

Components	Volume (20 µL/reaction)
ReadiScript™ RT Reaction Mix (5X)	5 µL
RNA	1-5 µL (Up to 1 µg)
RNase-Free Water	Up to 20 µL

2. Carefully mix the reagents with a gentle vortex followed by a brief centrifuge.
3. Incubate the reaction in a thermal cycler at 65°C for 5 minutes.
4. Cool the reaction at room temperature to allow the primers to anneal to the DNA (approximately 5 minutes).
5. Add 1 µL of ReadiScript™ RT Enzyme to the reaction.
6. Carefully mix the reagents with a gentle vortex followed by a brief centrifuge.
7. Incubate the reaction in a thermal cycler at 25°C for 5 minutes.
8. Incubate the reaction in a thermal cycler at 42°C for 60 minutes.
9. Terminate the reaction by incubating the reaction at 65°C for 10 minutes.
10. Place the completed cDNA synthesis reaction on ice for use in downstream applications. For long-term storage, the reaction can be stored at -20 °C.

EXAMPLE DATA ANALYSIS AND FIGURES

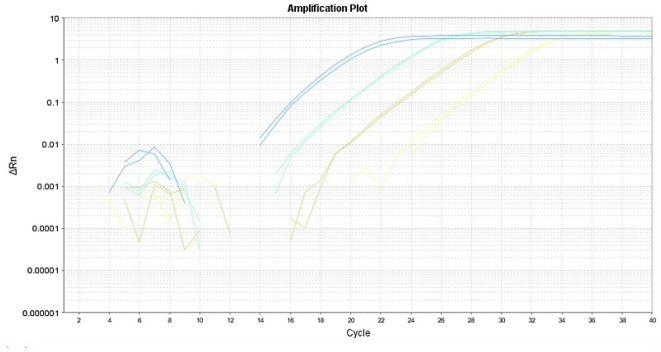

Figure 1. GAPDH gene expression analysis using TAQuest™ qPCR Master Mix with Helixyte™ Green *Low ROX*. Total RNA was extracted from HeLa cells, and cDNA was amplified in replicate reactions (1000, 100, 10, 1, 0 ng- From left to right) using the ReadiScript™ RT Reverse Transcription Kit. 2 µL volumes were used for each reaction for the qPCR detection of GAPDH.

DISCLAIMER

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