cDNA synthesis for use in RT-PCR

Protocol taken from: Quantitect Reverse Transcription Handbook 03/2009

Principle

This protocol uses the QuantiTect Reverse Transcription Kit available from Qiagen.
This is a fast and convenient procedure for cDNA synthesis with integrated genomic DNA removal. Genomic DNA contamination in RNA samples is effectively eliminated by gDNA Wipeout Buffer. All components that are required for fast and efficient reverse transcription are provided with the QuantiTect Reverse Transcription Kit, including Quantiscript Reverse Transcriptase, Quantiscript RT Buffer, and a unique RT Primer Mix. The synthesized cDNA is optimized for use in real-time PCR, allowing reliable quantification of targets from all regions of mRNA transcripts.

Protocol

Please refer to the protocol described in the QuantiTect Reverse Transcription Handbook: (http://www.qiagen.com/knowledge-and-support/resource-center/resource-download.aspx?id=f0de5533-3dd1-4835-8820-1f5c088dd800&lang=en)

Protocol Modification:

After "Step 7" (Incubation at 95°C to inactivate Quantiscript Reverse Transcriptase), the 20 µL cDNA solution is split into two 10 µL aliquots:

- 10 µL is stored at -20°C
- 10 µL is diluted 8x (add 70 µL RNase-free water). This is the solution which will be used for the PCR-analysis