Concentration:

RTScript™ cDNA Transcriptase: 200Units/uL
RTScript™ Reaction Buffer: 5X

Storage and Handling:

Store at -20°C upon arrival until provided expiration date. Minimize Freeze Thaw to avoid loss of performance.

Product Description:

RTScript™ cDNA Transcriptase:
Modified version of M-MLV Reverse Transcriptase with RNase H activity deactivated and increased thermal stability. In combination with optimized buffers and reagents, Empirical's RTScript™ enzyme synthesizes complementary DNA from RNA initiated from RNA or DNA Primers. Empirical's RTScript™ is sensitive, specific, and is capable of synthesizing highly structured and long cDNA fragments. Produced cDNA is appropriate for both PCR and qPCR analysis. RTScript™ cDNA Transcriptase is supplied with 5X RTScript™ Reaction Buffer and 100mM DTT.

Protocol:

Step 1A: cDNA synthesis without denaturation.
General guidelines per 20µl reaction for setup without sample denaturation include:

Component	Stock Conc.	Final Conc.	20uL Assay
RNase-free water	-	-	Up to 20µL
RNA template	-	Total RNA: 10 pg-5 µg or mRNA: 10pg-500ng	X µL
Primer	100µM	Gene specific primer: 10-20pg (50-100ng) Oligo-dT_{20} primer or random: 50pmol	0.1-0.2 µL 0.5µL
5X RTScript™ Reaction	5X	1X	4µL
dNTP Mix	10mM	500µM each	1 µL
DTT stock solution	100mM	5mM	1 µL
RNase Inhibitor	40 units / µL	20 units	0.5 µL
RTScript™ Reverse Transcriptase	200 units / µL	100 units	0.5 µL

Step 1B (optional): cDNA synthesis with denaturation
First, prepare the Template/Primer mix using the table below. Incubate the mix for 5 min at 65-70°C.

Component	Stock Conc.	Final Conc.	20uL Assay
RNase-free water	-	-	Up to 10µL
RNA template	-	Total RNA: 10 pg-5 µg or mRNA: 10pg-500ng	X µL
Primer	100µM	Gene specific primer: 10-20pg (50-100ng) Oligo-dt_{15-25} primer or random: 50pmol	0.1-0.2 µL 0.5µL

****This product is intended for Research Use Only. This product is manufactured under ISO13485:2016 Quality System Requirements and is available for use as a Raw Material for use in IVD applications. Please contact Empirical Bioscience for further details.**
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Second, prepare a Reaction mix using the table below.

Component	Stock Conc.	Final Conc.	20uL Assay
RNase-free water	-	-	Up to 10µL
5X RTScript™ Buffer	5X	1X	4µL
dNTP Mix	10mM	500µM each	1 µL
*DTT stock solution	100mM	5mM	1 µL
**RNase Inhibitor	40 units / µL	20 units	0.5 µL
***RTScript™ Reverse Transcriptase	200 units / µL	100 units	0.5 µL

*Adding up to 5mM DTT may increase the yield and is recommended for individual optimization **Addition of 20-40 Units of RNase inhibiter per assay is recommended when using low amounts of starting RNA. ***100 Units of enzyme is recommended for standard assays, but increased transcription levels may be achieved with increasing the amount of enzyme up to 200 units.

Third, Add 10µl of Reaction Mix to 10uL of Template/primer mix and pipette gently up and down on ice.

Step 2: Incubation
Gene Specific primers: Incubate Reaction Mix for 30-60min at 50°C.
Oligo-dT or Random primers: Incubate Reaction Mix for 10min at 42°C followed by 30-60min at 50°C.

Step 3 (optional): Heat inactivation
Heat the mixture for 70°C for 10 min to inactivate Reverse Transcriptase.

Step 4 (optional): RNA removal
Add 2 Units DNase-free RNase and incubate at 37°C for 20min. The cDNA can now be used as a template in PCR and should be stored at -20°C.

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