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	PIS-049 RT-CSK Reactions	Version: 004 Effective Date: 07/06/20 Author: Beth Lowe CO#: 062520-1

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Concentration:

RTScript Reverse Transcriptase: 200 units/uL, RTScript Buffer: 5X, dNTP Mix: 10mM, DTT Solution: 100mM, Oligo-(dT)20 primer: 100µM, Random Hexamers: 100µM, Rnase Inhibitor: 40 Units/uL, Positive Control RNA: 10ng/uL

Storage and Handling:

Upon arrival store at -20°C for provided expiration date. Minimize Freeze thaw to avoid loss of performance.

Ordering Information:

Item Number	Number of Tubes and Volumes	Number of 20uL Reactions
RT-CSK-100	RTScript Reverse Transcriptase, 200 units/uL: 1x100µL, 5X RTScript Buffer: 1x400µL, dNTP Mix, 10mM: 1x100µL, DTT Solution, 100mM: 1x100µL, Oligo-(dT)20 primer, 100µM: 1x50µL, Random Hexamers, 100µM: 1x50µL, Rnase Inhibitor, 40 Units/uL: 1x10µL, Positive Control RNA, 10ng/uL: 1x10µL, Rnase-free Water - 1x1.2mL	100 Reactions

Product Description:

Empirical's RTScript™ cDNA Synthesis Kit contains all the components necessary to perform first strand cDNA synthesis with high sensitivity and efficiency. RTScript™ cDNA Synthesis Kit is a 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 single stranded RNA or DNA templates. 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.

Empirical RT Kit is supplied with RTscript™ Reverse Transcriptase (200U/µL), 5X RTScript™ Buffer, dNTP Mix (10mM), DTT Stock Solution (100mM), Oligo-(dT)20 primer (100µM), Random Hexamers (100µM), RNase Inhibitor (40 units/µL), Positive Control RNA (10ng/µL), and RNase-free Water.

Protocol: Minimize Freeze thaw of master mix to avoid loss of performance. The following reaction set up and general cycling conditions are recommended but can vary depending on the template and primers being used.

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	Not Applicable	Not Applicable	Up to 20µL
RNA template	Not Applicable	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™ 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

**** 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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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	Not Applicable	Not Applicable	Up to 10µL
RNA template	Not Applicable	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

- Second, prepare a Reaction mix using the table below. Add 10uL of the denaturation mix to the reaction mix.

Component	Stock Conc.	Final Conc.	20uL Assay
RNase-free water	Not Applicable	Not Applicable	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 inhibitor 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.

The cDNA can now be used as a template in qPCR.

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