Total-Transcriptome cDNA Synthesis Kit

Store at -20°C

Part No.	Components	G904	G905
E017-1	Poly(A) Polymerase, Yeast (1U/µl)	12.5 µl	50 µl
E017-2	5X Poly(A) Polymerase Reaction Buffer	60 µl	240 µl
E017-3	25 mM $MnCl_2$	200 µl	200 µl
E017-4	ATP (10 mM)	40 µl	160 µl
RT-2	OneScript® Plus RTase (200 U/µl)	25 µl	100 µl
RT-15	Oligo (dT) adapter (10 µM)	40 µl	160 µl
RT-4	Random Primers (10 µM)	40 µl	160 µl
RT-5	dNTPs (10 mM)	40 µl	160 µl
RT-6	RNaseOFF Ribonuclease Inhibitor (40 U/µl)	15 µl	60 µl
RT-7	5X RT Buffer	150 µl	600 µl
RT-0	Nuclease-free H_2O	1 ml	2 x 1 ml
RT-16	Universal 3′ Reverse Primer (10 µM)	250 µl	1 ml
RT-17	GAPDH mRNA qPCR Control Assay (10 µM)	50 µl	200 µl
RT-18	miR-16 miRNA qPCR Control Assay (10 µM)	50 µl	200 µl
RT-19	Y lncRNA qPCR Control Assay (10 µM)	50 µl	200 µl
	Size	**25 rxns**	**100 rxns**

Product Description

Total-Transcriptome cDNA Synthesis Kit contains all materials required for a complete first-strand cDNA synthesis from all RNA, including non-coding RNAs; featuring the use of poly(A) polymerase and OneScript® Plus RTase. **Poly(A) Polymerase** catalyses the template independent addition of adenosine residues onto the 3′ ends of polyribonucleotides. All non-coding RNAs and smaller RNAs, such as miRNAs, after being poly(A)-tailed can be reverse-transcripted via the use of oligo d(T). **OneScript® Plus Reverse Transcriptase** is a novel recombinant reverse transcriptase that exhibits much higher efficiency in the first-strand cDNA synthesis from RNA templates with secondary structures and high GC content. It is engineered to perform under high temperatures (50°C - 55°C), facilitating the elimination of secondary structures associated with GC-rich RNA templates.

Storage Conditions

Store all components at -20°C in a non-frost-free freezer. All components are stable for 1 year from the date of shipping when stored and handled properly.

Protocol

Reverse transcription reactions should be assembled in an RNase-free environment. The use of "clean", automatic pipettes designated for PCR and aerosol-resistant barrier tips are recommended.

A. cDNA Synthesis:

1. Thaw RNA templates and all reagents on ice. Mix each solution by vortexing gently.
2. Prepare the following Poly(A) Polymerase tailing reaction mixture on ice.

Components	Volume	Final Concentration
Total RNA	Variable	1 ng - 2 µg/rxn
5X Poly(A) Polymerase Reaction Buffer	2 µl	1X
ATP (10 mM)	1.5 µl	1.5 mM
25 mM $MnCl_2$	1 µl	2.5 mM
Poly(A) Polymerase, Yeast (1U/µl)	0.5 µl	0.5 U/rxn
Nuclease-free H_2O	To 10 µl	-

3. Incubate the mixture at 37°C for 30 mins; centrifuge briefly to collect content and add the following to the poly(A)-tailed reaction mixture.

Components	Volume	Final Concentration
Oligo (dT) adapter (10 µM)	1.5 µl	0.75 µM

4. Heat mixture to 65°C for 5 mins and incubate on ice for at least 1 min. Collect all components by a brief centrifugation.
5. Add the following to the reaction mixture:

Components	Volume	Final Concentration
5X RT Buffer	4 µl	1X
Random Primers (10 µM)	1.5 µl	0.75 µM
dNTP Mix (10 mM each)	1.5 µl	750 µM
RNaseOFF Ribonuclease Inhibitor (40 U/µl)	0.5 µl	20 U/rxn
OneScript® Plus RTase (200 U/µl)	1 µl	200 U/rxn

6. Mix components well and collect all components (20 µl) by a brief centrifugation.
7. Perform cDNA synthesis by incubating the tube for either 15 mins (for qPCR) or 50 mins (for PCR) at 50°C.
8. Stop reaction by heating it at 85°C for 5 mins. Chill on ice. The newly synthesized first-strand cDNA is ready for immediate downstream applications, or for long-term storage at -20°C.

B: qPCR Quantification:

1. Prepare the following qPCR reaction mixture:

Components	Volume	Final Concentration
2X qPCR MasterMix (Not Included) Note 1	10 µl	1X
Total-Transcriptome cDNA from Part A Note 2	0.5 - 1 µl	≤500 ng/rxn
qPCR Control Assay (10 µM) Note 3	0.6 µ	300 nM
- - - - - - - - - - or - - - - - - - - - -	- - - - or - - - -	- - - - - - or - - - - - -
Specific 5′ Forward Primer (10 µM)	0.6 µl	300 nM
Specific/Universal 3′ Reverse Primer (10 µM)	0.6 µl	300 nM
Nuclease-free H_2O	To 20 µl	-

General Notes

1. Consider using EvaGreen miRNA qPCR Mastermix for all miRNA and lncRNA experiments; and consider using EvaGreen 2X qPCR MasterMix for all mRNA experiments with gene-specific forward and reverse primers.
2. cDNA dilution guideline: 100X dilution for 0.1 - 1 µg RNA used / 1000X dilution for > 1 µg RNA used.
3. Control Assays contain a specific forward primer, and a specific reverse primer (Part No. RT-17) or a 3′ universal reverse primer (Part No. RT-18 and Part No. RT-19).
4. To remove RNA complementary to the cDNA, add 1 µl (2 U) of *E. coli* RNase H and incubate at 37°C for 20 mins.

All crb PCR, RT-PCR, and qPCR products are ISO 13485:2003 and 13485:2012 certified as diagnostic grade and in compliance with all regulatory requirements for the design and manufacture of medical devices, as outlined by the International Organization for Standardization (ISO). For technical questions, please email us at support@coderegenesis.com or visit our website at www.coderegenesis.com.

• support@coderegenesis.com • www.coderegenesis.com

CODEREGENESIS