AccuPower® RocketScript™ RT Master Mix (V0/2019-08-01)

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I. Introduction

AccuPower® RocketScript™ RT Master Mix contains all components for first-strand cDNA synthesis from purified Poly(A) or total RNA template.

The *AccuPower® RocketScript™* RT Master Mix contains *RocketScript* Reverse Transcriptase, a new *M-MLV* originated Reverse Transcriptase that has been engineered to provide increased thermal stability in order to synthesize full length first-strand cDNA more efficiently. The amount of starting material can vary from 1 pg to > 1 µg of total RNA and RNA targets from 100 bp to > 10 kb can be detected with the *AccuPower® RocketScript™* RT Master Mix.

The *AccuPower® RocketScript™* RT Master Mix can be used to synthesize cDNA at a temperature range of 42 – 70°C, providing increased specificity, higher yields of cDNA, and more full-length product than other reverse transcriptases.

II. Application

- Standard RT and RT-PCR
- Real-Time PCR
- Synthesis of double-stranded cDNA for cloning
- Gene expression level analysis

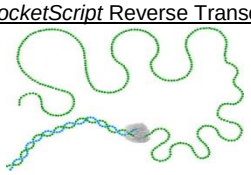
III. Contents

Component	Amount
RocketScript Reverse Transcriptase	200 U
5 x Reaction Buffer	1 x
DTT	0.25 mM
dNTP	250 µM each
RNase Inhibitor	1 U

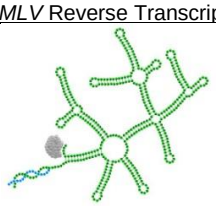
IV. Principle

RocketScript Reverse Transcriptase in the *AccuPower® RocketScript™* RT Master Mix is genetically engineered thermal stable *M-MLV* Reverse Transcriptase with enhanced thermal stability and outstanding processivity. The enzyme also features increased specificity and improved efficiency allowing efficient reverse transcription of RNA molecules with complex secondary structures.

RocketScript Reverse Transcriptase at 70°C



M-MLV Reverse Transcriptase at 42°C



Competitor I at 50°C

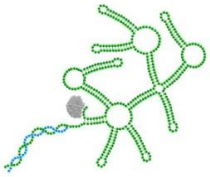


Figure 1. Schematic representation of the 5'UTR of a gene, with complex secondary structure, at three different temperatures.

Note that *RocketScript* shows full activity at 70°C allowing it to synthesize the complete gene sequence where *M-MLV* and other Reverse Transcriptase's fail.

V. Storage

AccuPower® RocketScript™ RT Master Mix should be stored at -20°C upon receipt and is stable until the expiry date stated on the label.

VI. Notice to Purchaser

- *AccuPower® RocketScript™* RT Master Mix exhibits RNase H activity.
- Bioneer corporation reserves the right to make corrections, modifications, improvements and other changes to its products, services, specifications or product descriptions at any time without notice. All information provided here is subject to change without notice.

VII. Additional Required Materials & Devices

- Thermal cycler for PCR
- Target-specific primers, oligo dT, random hexamer, or nonamers
- Calibrated micropipette
- Sterilized micropipette tips with filters

VIII. General Precautions

- Wear gloves during experiments to prevent contamination.
- Store positive materials, such as samples and control templates, in separated freezer from freezers for the kit.
- Add templates to the reaction mixture in clean bench or a spatially separated facility.

IX. Protocol

1. Thaw total RNA, DEPC-water, and primer before use.
2. Mix the total RNA and primer (oligo dT, random primer, or specific primer) in a sterile tube as indicated below.

Recommended amount of template and primer

Components	Amount
Total RNA	10 pg – 5 µg
Oligo dT	50 pmoles
Random primer(dN6,dN12)	100 pmoles
Specific primer	10 – 50 pmoles

3. Fill up the reaction volume with DEPC-water.
4. Mix the reaction mixture by vortexing or pipetting and briefly spinning down.
5. Perform the reaction under the following conditions.

Step	Temperature				Time
	dN_6	dN_{12}	dT_{20}	Specific primer	
Primer annealing	15°C	30°C	37°C	Tm of primer	10 min
cDNA synthesis	42°C				30 min
Heat inactivation	95°C				5 min

Alternative protocol

Step	Temperature	Time
cDNA synthesis	42~70°C	1 hr
Heat inactivation	95°C	5 min

Note: for difficult or high GC-content templates, use a 55°C cDNA synthesis temperature.

6. Maintain the reaction at 4°C after amplification. The sample can be stored at -20°C until use.

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X. Reaction Examples

1. Reaction mixture

Component	Volume	Amount
Template RNA	1 µl	100 pg
Oligo dT 20	1 ul	50 pmoles
RT Mastermix	10 ul	
DEPC-D.W	8 µl	
Total	20 µl	

2. Reaction condition

Step	Temperature	Time
Primer annealing (oligo dT20)	37°C	10 min
cDNA synthesis	60°C	1 hr
Heat inactivation	95°C	5 min

XI. Experimental Data

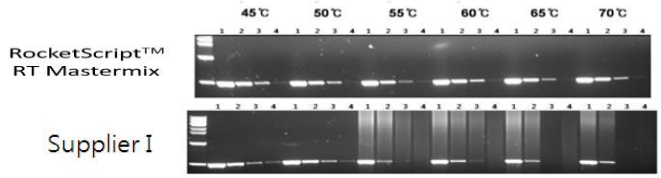

Figure 1. Amplification results of *AccuPower® RocketScript™* RT Master Mix using myc compared with Company I reverse transcriptase.
Reverse transcription condition: Incubation at each temperature 45, 50, 55, 60, 65, 70°C for 1 hr, inactivation at 95°C for 5 min / Primer set: human myc 495 bp set

Lane M: 1 kb DNA Ladder
Lane 1: 100 ng Human total RNA from HeLa cell
Lane 2: 10 ng Human total RNA from HeLa cell
Lane 3: 1 ng Human total RNA from HeLa cell
Lane 4: 100 pg Human total RNA from HeLa cell

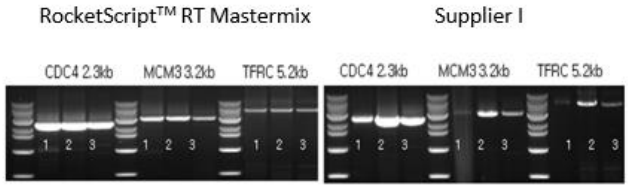

Figure 2. Comparison of long kb amplification between *AccuPower® RocketScript™* RT Master Mix and Company I reverse transcriptase.
Reverse transcription reactions were performed according to each manufacturer's recommendation. All cDNAs were amplified with *AccuPower®* HotStart PCR Master Mix (K-5050) from Bioneer. Note supplier I shows inhibition with high input concentration of total RNA.

Lane 1: 1 µg Human total RNA from HeLa cell
Lane 2: 100 ng Human total RNA from HeLa cell
Lane 3: 10 ng Human total RNA from HeLa cell

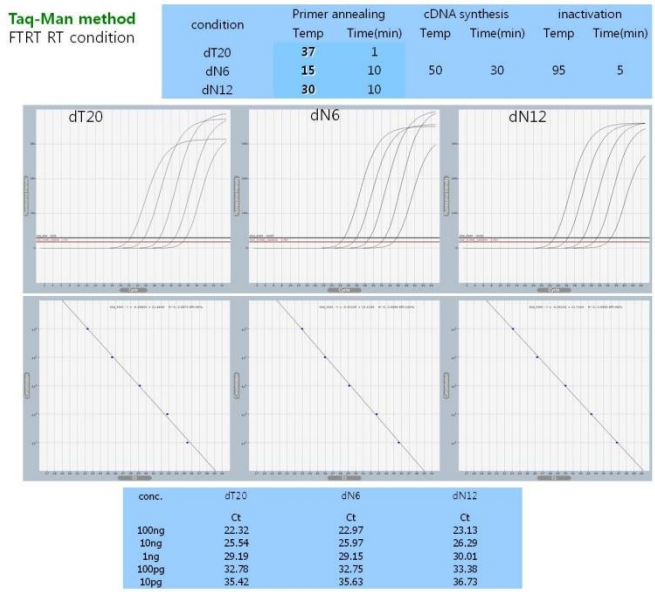

Figure 3. Performance on Comparison oligo (Oligo dT 20, dN 6, dN 12)
Template RNA: Human total RNA in Hela cell
Concentration 0.01 ng ~ 100 ng, 10 fold serial dilution.

XII. Notice

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XIII. Ordering Information

Cat. No.	Description
K-2105	*Accupower® RocketScript™* RT Master Mix (2X, 1 ml)

A complete product list appears on our web site at *www.bioneer.com*