AccuPower® RocketScript™Cycle RT PreMix(dN12)

(V2/2018-04-13)

Bioneer Corporation
8-11 Munpyeongseo-ro, Daedeok-gu, Daejeon, 34302
Republic of Korea
Tel: +82-42-930-8777 (Korea : 1588-9788)
Fax: +82-42-930-8688 E-mail: sales@bioneer.com

Bioneer Inc.
155 Filbert St. Suite 216
Oakland, CA 94607, USA
Toll Free: +1-877-264-4300 Fax: +1-510-865-0350
E-mail: order.usa@bioneer.us.com

Bioneer R&D Center
Korea Bio Park BLDG #B-702, 700 Daewangpangyo-ro
Bundang-gu, Seongnam-si, Gyeonggi-do, 13488
Republic of Korea
Tel: +82-31-628-0500 Fax: +82-31-628-0555

I. Introduction

AccuPower® RocketScript™ Cycle RT PreMix(dN12) is a ready-to-use vacuum dried mastermix containing all components for first-strand cDNA synthesis from purified Poly(A) or total RNA template. Random Primer $(dN)_6$ is also included in the premix for added convenience. Simply add your template and DEPC-water to begin your reaction.

The *AccuPower RocketScript* Cycle RT PreMix(dN12) 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* Cycle RT PreMix(dN12).

The *AccuPower RocketScript* Cycle RT PreMix(dN12) 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

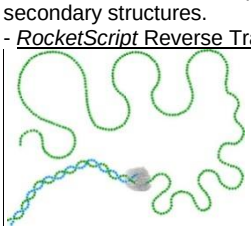
III. Contents

Component	Amount
RocketScript Reverse Transcriptase	200 U
Random Primer $(dN)_6$	100 pmole
5 x Reaction Buffer	1 x
DTT	0.25 mM
dNTP	250 µM each
RNase Inhibitor	1 U

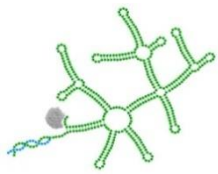
IV. Principle

RocketScript Reverse Transcriptase in the *AccuPower RocketScript* Cycle RT PreMix(dN12) 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

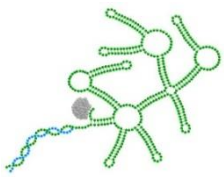


Figure 1. Schematic representation of the 5'UTR of a gene, with complex secondary structure, at three different temperatures. Note that *RocketScript* Reverse Transcriptase shows full activity at 70°C allowing it to synthesize the complete gene sequence where *M-MLV* and other Reverse Transcriptases fail.

V. Storage

AccuPower RocketScript Cycle RT PreMix(dN12) 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* Cycle RT PreMix exhibits RNase H activity and contains Random Primer $(dN)_{12}$
- 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
- 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 and DEPC-water before use.
2. Add 10 pg – 5 µg of total RNA into the *AccuPower RocketScript* Cycle RT PreMix tubes.
3. Add DEPC-water into the *AccuPower RocketScript* Cycle RT PreMix tubes to a total volume of 20 µl (K-2208, K-2209) or 50 µl (K-2210). Do not calculate the dried pellet.
4. Dissolve the vacuum dried pellet completely and spin down by using Bioneer's *ExiSpin* Vortex/Centrifuge or by pipetting up and down several times and briefly spinning down.
5. Perform the reaction under the following conditions.

*Cyclic Temperature Reverse Transcription (CTRT) method option A

Step	Temperature	Time	No. of Cycles
Primer annealing	15°C	20 sec	> 10cycle
cDNA synthesis	50°C	4min	
Denaturation of RNA secondary structure and cDNA synthesis	60°C	20 sec	
Heat inactivation	95°C	5 min	1

*Cyclic Temperature Reverse Transcription (CTRT) method option B

Step	Temperature	Time	No. of Cycles
Primer annealing	15°C	1 min	> 10
cDNA synthesis	42 – 70°C	4 min	
Heat inactivation	95°C	5 min	1

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.

X. Reaction Example

1. Reaction mixture

Component	Volume	Concentration
Template RNA	1 µl	100 pg/µl
DEPC-D.W	19 µl	
Total	20 µl	

2. Reaction condition

Step	Temperature	Time	No. of Cycles
Primer annealing	15°C	20 sec	> 10
cDNA synthesis	50°C	4 min	
Denaturation of RNA secondary structure and cDNA synthesis	60°C	20 sec	
Heat inactivation	95°C	5 min	1

Copyright 2018. Bioneer Corporation. All Rights Reserved.

Bioneer Corporation
8-11 Munpyeongseo-ro, Daedeok-gu, Daejeon, 34302
Republic of Korea
Tel: +82-42-930-8777 (Korea : 1588-9788)
Fax: +82-42-930-8688 E-mail: sales@bioneer.com

Bioneer Inc.
155 Filbert St. Suite 216
Oakland, CA 94607, USA
Toll Free: +1-877-264-4300 Fax: +1-510-865-0350
E-mail: order.usa@bioneer.us.com

Bioneer R&D Center
Korea Bio Park BLDG #B-702, 700 Daewangpangyo-ro
Bundang-gu, Seongnam-si, Gyeonggi-do, 13488
Republic of Korea
Tel: +82-31-628-0500 Fax: +82-31-628-0555

XI. Experimental Data

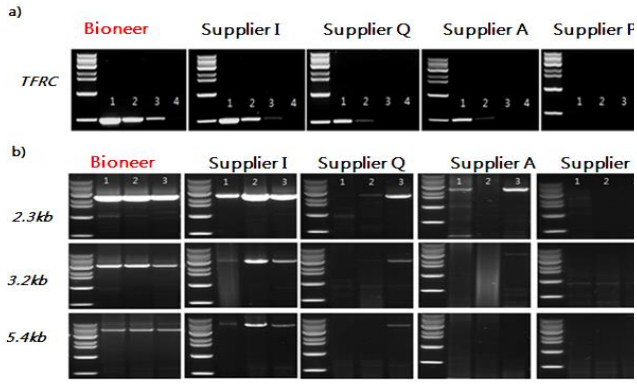


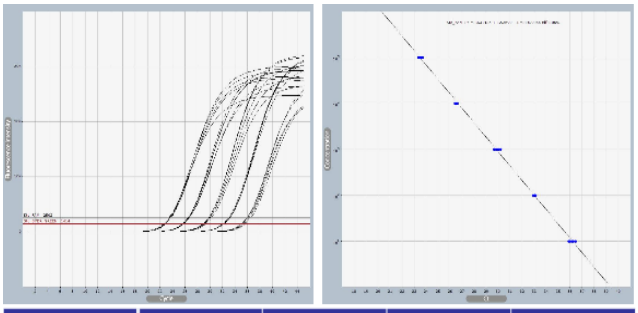
Figure 1. Comparison of amplification efficiency between *AccuPower RocketScript* Cycle RT PreMix and competitors' RTases

(a) Sensitivity test
Primer set: Human TFRC set
Lane M: 1 kb DNA Ladder
Lane1: 100 ng Human total RNA from HeLa cell
Lane2: 10 ng Human total RNA from HeLa cell
Lane3: 1 ng Human total RNA from HeLa cell
Lane4: 100 pg Human total RNA from HeLa cell
RT reaction condition is performed according to each manufacturer's recommendations

(b) Full-Length cDNA synthesis test
RT reactions were performed according to each manufacturer's recommendation. All cDNAs were amplified with *AccuPower* Hotstart PCR Premix (K-5050) from Bioneer
Note: Competitor products show inhibition at high input concentrations 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 2. Comparison of amplification results between Bioneer *RocketScript*™ Cycle RT PreMix and competitor RTases

All cDNAs were amplified with *AccuPower* Hotstart PCR Premix (K-5050) from Bioneer
Primer set: human Myc498 bp set
Lane M: 1 kb DNA Ladder
Lane 1: 10 ng Human total RNA from HeLa Cell
Lane 2: 1 ng Human total RNA from HeLa Cell
Lane 3: 100 pg Human total RNA from HeLa Cell
Lane 4: 10 pg Human total RNA from HeLa Cell



dN6	Primer annealing		CDNA synthesis		Denaturation of RNA secondary structure		inactivation	
	Temp	Time	Temp	Time	Temp	Time	Temp	Time
FTRT 1cycle	15	10	42	30			95	5
CTRT3/5/7/10cycle	15	1	42	4	60	20		

dN6	FTRT	CTRT with 3cycle	CTRT with 5cycle	CTRT with 7cycle	CTRT with 10cycle
Efficiency	99	96	95	99	101
Linearity	0.9981	0.998	0.9985	0.9997	0.9993
	Ct	Ct	Ct	Ct	Ct
100ng	23.36	23.59	23.48	23.56	23.68
10ng	26.41	26.45	26.43	26.60	26.52
1ng	29.67	29.73	29.91	30.01	30.20
100pg	33.09	32.93	33.12	33.08	33.09
10pg	36.14	36.25	35.87	35.96	36.46

Figure 3. Low copy species enrichment by cycle.

Comparing FTRT (Fixed Temperature Reverse Transcription) to 3 and 10 cycle(s) of CTRT reveal progressive improvement in detected cDNA yield as input copies decrease. FTRT: 30 min incubation at 42°C followed by 5 min deactivation at 95°C CTRT: Cycles of 15°C annealing 1min, 50°C cDNA synthesis 4 min, 60°C secondary structure melting and cDNA synthesis 20 sec. All cDNAs were amplified with AccuPower® DualStar™ qPCR PreMix (K-6110) from Bioneer.

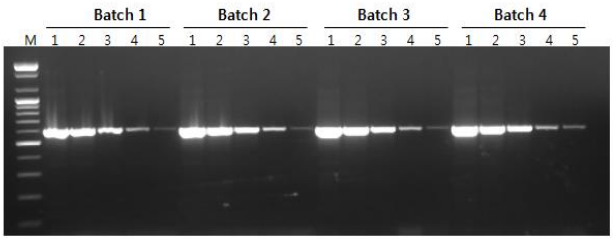


Figure 4. Highly reproducible Amplifications

Amplification of an 600bp target gene was detected using Human total RNA (from 100ng to 10pg) with AccuPower RocketScript Cycle RT PreMix(dN12).
As shown in Fig 4. Highly reproducible Amplifications were achieved within each Lot.
Lanes 1-5: 100 ng, 10 ng, 1 ng, 100pg and 10pg of total RNA from HeLa cells, respectively.

XII. Ordering Information

Cat. No.	Description
K-2208	*AccuPower RocketScript* Cycle RT PreMix(dN12), 20 µl, 0.2 ml thin-wall 8-strip tubes with attached cap, 96 tubes
K-2209	*AccuPower RocketScript* Cycle RT PreMix(dN12), 20 µl, 0.2 ml thin-wall 8-strip tubes with attached cap, 480 tubes
K-2210	*AccuPower RocketScript* Cycle RT PreMix(dN12), 50 µl, 0.2 ml thin-wall 8-strip tubes with attached cap, 96 tubes

Visit us at www.bioneer.com to learn about Bioneer products and services.

Copyright 2018. Bioneer Corporation. All Rights Reserved.