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AccuPower® CycleScript RT PreMix (dN<sub>12</sub>) is a ready-to-use reverse transcription kit, which can generate homogeneous cDNA synthesis through temperature cycling (patent pending). This product contains all components including thermostable CycleScript Reverse Transcriptase, dNTPs, reaction buffer, primer, and stabilizers for reverse transcription and is stable for 2 years at -20°C. AccuPower® CycleScript RT PreMix (dN<sub>12</sub>) has high reverse transcription activity in broad ranges of temperature between conventional 42°C and 55°C. This product is designed for Cyclic Temperature Reverse Transcription (CTRT), which the CTRT reaction can be performed in higher performance than that of reverse transcription reaction at conventional single temperature.

The CTRT reaction is composed of 2 or 3 steps as follows; The Step 1 is performed at  $15\sim25^{\circ}$ C, at which short primer is fully annealed. And then, the Step 2 is performed at  $42\sim48^{\circ}$  (optional) for cDNA synthesis. The Step 3 is performed at high temperature  $50\sim55^{\circ}$ C at which secondary structure of RNA template obstructing reverse transcription is melted and reverse transcription is also occurred.

## Advantages

- Speed: Substantial reduction in reaction setup time. No need adding primer and RNA template denaturation step. No difference between with and without denaturation step.
- Stability: As each tube of AccuPower<sup>®</sup> CycleScript RT PreMix (dN<sub>12</sub>) contains a stabilizer (patented in US and Korea), which can maintain the stability of the CycleScript reverse transcriptase up to 2 years at -20°C.
- Reproducibility: The strict functional QC assays demonstrated highly consistent and reproducible RT performance. In most applications an increase in yield is observed as compared to the standard reactions.
- Simplicity: The fewer manual steps allow reduction in potential errors and cross contaminations. Just add RNA template and DEPC DW.

### **Experimental Protocol**

- 1. Add the RNA template. Any kinds of RNAs are available.
- Recommended concentration: 0.1~1.0 µg of Total RNA or 0.01~0.1 µg of Poly(A) RNA
- 2. Fill up to the  $20\mu$ l reaction volume with DEPC DW.
- Dissolve the vacuum-dried transparent pellet by vortexing or tapping, and briefly spin down. The pellet should be dissolved completely.
  Perform cDNA synthesis reaction either CTRT reaction or single temperature reaction.
  - cDNA synthesis  $\rightarrow$  RTase inactivation
  - 4-1) CTRT reactions (examples)

	CTRT 1				CTRT 2				
Step 1	15~25℃	30 sec : primer annealing		Step 1	15~25℃	1 min : primer annealing	Repeat		
Step 2	42~45℃	4 min : cDNA synthesis	Repeat 12 times		Step 2	42~50°C	4 min : melting secondary	12 times or less	
Step 3	55℃	30 sec : melting secondary	or less	structure & cDNA synthesis					
		structure & cDNA synthesis			Heat	95°C	5 min		
Heat inactivation	95℃	5 min			inactivation	90 C	511111		

4-2) Single temperature reaction : 37 ~ 50°C (You can choose one temperature but this product prefers 42~48°C reaction)

30 ~ 60 min. →95°C 5 min

If PCR is followed RT reaction, perform the PCR with AccuPower® PCR PreMix from Bioneer as follows :

1) Add an aliquot of 2~5 μl of the finished RT product (synthesized cDNA) to the *AccuPower*<sup>®</sup> PCR PreMix tube.

- 2) Perform PCR cycles according to the PCR condition.
- (Annealing temperature and time should be optimized according to each primer/template combination.)

### **Ordering Information**

Tube type	Reaction	Cat.No	Description	Tube type	Reaction	Cat.No	Description
0.2 ml Tube	20 µl	K-2044	dT20 / 0.2 ml thin-wall 8-strip tubes with attached cap / 96 tubes		20 µl	K-2046	dN <sub>6</sub> / 0.2 ml thin-wall 8-strip tubes with attached cap / 96 tubes
		K-2044-B	dT20 / 0.2 ml thin-wall 8-strip tubes with attached cap / 480 tubes	0.2 ml		K-2046-B	$dN_6$ / 0.2 ml thin-wall 8-strip tubes with attached cap / 480 tubes
	50 µl	K-2047	dT20 / 0.2 ml thin-wall 8-strip tubes with attached cap / 96 tubes	Tube	50 µl	K-2049	$dN_6$ / 0.2 ml thin-wall 8-strip tubes with attached cap / 96 tubes
		K-2047-B	dT20 / 0.2 ml thin-wall 8-strip tubes with attached cap / 480 tubes			K-2049-B	dN <sub>6</sub> / 0.2 ml thin-wall 8-strip tubes with attached cap / 480 tubes
	20 µl	K-2045	dN12 / 0.2 ml thin-wall 8-strip tubes with attached cap / 96 tubes		50 µl	K-2050	dT <sub>20</sub> / 0.5 ml thin-wall tubes with attached cap / 100 tubes
		K-2045-B	dN12 / 0.2 ml thin-wall 8-strip tubes with attached cap / 480 tubes	0.5 ml		K-2050-1	dN12 / 0.5 ml thin-wall tubes with attached cap / 100 tubes
	50 µl	K-2048	dN12 / 0.2 ml thin-wall 8-strip tubes with attached cap / 96 tubes	Tube		K-2050-2	$dN_{\rm 6}/0.5$ ml thin-wall tubes with attached cap / 100 tubes
		K-2048-B	$dN_{12}/0.2$ ml thin-wall 8-strip tubes with attached cap / 480 tubes				

#### Notice to Purchaser

This product is optimized for use in the CTRT covered by patent(pending) applied by Bioneer Corporation. No license under this patent to use CTRT Process is conveyed expressly or by implication to the purchaser by the purchase of this product.

This product is sold for research use only and is not to be administered to humans or used for medical diagnostics. Further information on purchasing licenses may be obtained by contacting the Director of Licensing at Bioneer Corporation.

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