

# AccuPower® Dual-HotStart™ RT-PCR PreMix (with UDG)

(V3/2018-04-12)

## I. Introduction

AccuPower® Dual-HotStart™ RT-PCR PreMix (with UDG) is a ready-to-use reagent containing all components necessary for RT-PCR except for template and target-specific primers. To start your reaction, simply add template RNA and primers specific to your gene of interest into a reaction vessel containing the vacuum dried PreMix. The PreMix is stable for 2 years at -20°C.

## II. Principle

AccuPower® Dual-HotStart™ RT-PCR PreMix (with UDG) uses a Dual-Hotstart RT-PCR technique that detects only the desired target gene.

### 1) Hotstart Reverse Transcription

The AccuPower® Dual-HotStart™ RT-PCR PreMix (with UDG) uses a unique enzyme-mediated Hotstart method that provides robust, sensitive, and reliable cDNA synthesis results. Bioneer's RocketScript™ reverse transcriptase is completely inhibited by pyrophosphate at temperatures below 50°C. However, RocketScript™ reverse transcriptase becomes fully active at temperatures above 50°C via pyrophosphate hydrolysis with a thermostable pyrophosphatase. This prevents the formation of mis-primed products and primer-dimers during the reaction set up process resulting in improved specificity of cDNA synthesis.

### 2) Hotstart Polymerase Chain Reaction

Bioneer's HotStart Taq DNA polymerase provides superior priming accuracy and specificity that cannot be achieved with other enzymes. You will use less enzyme per reaction, save money, and get higher sensitivity than with other hotstart enzymes.

### 3) UDG (Uracil DNA Glycosylase)

AccuPower® Dual-HotStart™ RT-PCR PreMix (with UDG) contains uracil DNA glycosylase, dA, dG, dC, dT with dUTP and reaction buffer in a premixed format that is vacuum dried into individual tubes. Carryover contamination is a significant source of error when PCR is being used in a diagnostic context. Use of uracil DNA glycosylase, which catalyzes the hydrolysis of N-glycosylic bond between the uracil and sugar, is a solution for this problem. Heating at 94°C leads to degrade contaminants (uracil-containing DNA) of PCR reaction mixture. UDG efficiently remove uracil from single-stranded or double-stranded DNA, but from oligomers (6 or fewer). It is not active for targeting RNA or uracil-free DNA.

## III. Storage

For long term storage, AccuPower® Dual-HotStart™ RT-PCR PreMix (with UDG) should be stored at -20°C upon receipt and is stable until the expiry date stated on the label.

## IV. Content

Cat. No	Size	Descriptions
K-6714	96 tests	AccuPower® Dual-HotStart™ RT-PCR PreMix (with UDG), 0.2 ml thin-wall 8-Strip tubes with attached cap, 20 µl/rxn
K-6715	96 tests	AccuPower® Dual-HotStart™ RT-PCR PreMix (with UDG), 0.2 ml thin-wall 8-Strip tubes with attached cap, 50 µl/rxn

## V. General Precautions

- Wear gloves during experiments to prevent contamination.
- Store positive materials, such as samples and control templates, in a separate freezer from the kit.

## VI. Additional Required Materials & Devices

- Thermal Cycler for PCR (authorized instruments)
- Target-specific primers
- Calibrated micropipette, Vortex mixer
- Sterilized micropipette tips with filters

## VII. Protocol

### [ 20 µl reaction volume ]

- Thaw Total RNA, DEPC-water and Specific primer before use.
- Add Total RNA and Specific primer into AccuPower® Dual-HotStart™ RT-PCR PreMix (with UDG) tubes.

Components		Amount
Template RNA	Total RNA	10 µg ~5 µg
	Poly(a) RNA	10 µg ~5 µg
Specific Primer		10-30 pmoles

- Add DEPC-water into AccuPower® Dual-HotStart™ RT-PCR PreMix (with UDG) tubes to a total volume of 20 µl. Do not calculate the dried pellet.

- Dissolve the vacuum dried pellet by flick with your finger or pipetting, and briefly spin down.

- Perform the reaction under the following conditions.

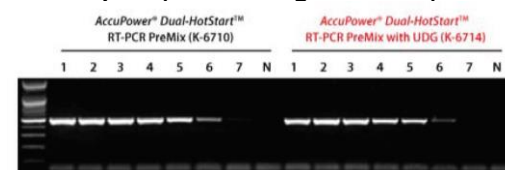
Step	Temperature	Time	Cycles
UDG activation	37°C	2 min	1
cDNA synthesis	42-70°C	10-60 min	1
Pre-Denaturation	95°C	10 min	1
Denaturation	95°C	10-30 sec	30-40
Annealing	50-65°C	10-30 sec	
Extension	72°C	1kb/1 min	
Final extension	72°C	5 min	1

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

Note: reaction temperature should be selected to fit the Tm value of Primers

## VII. Experimental Data

### RNA template (not including uracil base)



### RNA template (including uracil base)

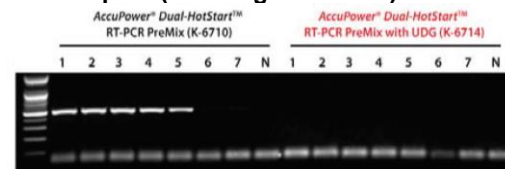


Figure 1. Comparison of amplification quality using PCR products (not including uracil base or including uracil base) between AccuPower® Dual-HotStart™ RT-PCR PreMix and AccuPower® Dual-HotStart™ RT-PCR PreMix (with UDG).

## VIII. Notice

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.