Product Instruction

【Product Name】

Product Name：AK Taq One-Step Enzyme Mix With UDG（Plus）

Product No：MDE013-6

【Product Description】

AK Taq One-Step Enzyme Mix With UDG（Plus） is designed for one-step RT-qPCR, a reverse transcription protocol in which cDNA synthesis and PCR amplification steps are performed in a single reaction, minimizing risk of contamination. This enzyme mix combines several premium Fapon's enzymes to create a robust reverse transcription product that delivers high-throughput reaction capacity through the utilization of Fapon's highly sensitive reverse transcriptase and the convenience of hot-start Taq DNA polymerase. AK Taq DNA Polymerase is a thermostable DNA polymerase variant of Taq DNA Polymerase which has been engineered to have enhanced binding ability to DNA substrates, enabling amplification of PCR templates in the presence of inhibitors. The enzyme mix incorporates hot start technology which allows for greater flexibility during reaction preparation by decreasing primer-dimer formation and limiting nonspecific amplification at ambient temperatures. Additionally, the enzyme mix includes uracil DNA glycosylase (UDG) which allows for elimination of carryover contamination from the laboratory environment.

This enzyme mix is best suited for real time RT-qPCR detection of trace RNA in minimally pre-treated RNA virus samples. In addition, this product is compatible with rapid PCR conditions in our optimized buffer system. The product can also be lyophilized as a master mix for your convenience after adding our optimized buffer, protective agents for freeze-drying, and customer-provided primer-probe mix.

【Product content】

AK Taq One-Step Enzyme Mix With UDG (Plus)

5×AK Taq One Step RT-PCR Buffer

【Storage conditions】

Stored at -15℃ to -25℃ for one year.

【Product application】

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【Unit definition】

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【Announcements】

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【Other announcements】

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【Application method】

a.Thaw and mix well all reagents and put on ice. Aliquot PCR reagents to avoid extensive freeze-thaw.

b.Keep prepared reactions on ice before transferring to the PCR machine to prevent non-specific amplification.

c.Prepare PCR reactions on ice as directed in the table below (20 µL reaction). Reaction volume can be increased to 50 µL if desired amplification is not achieved in a 20 µL reaction.

d.According to the number of reactions, generate a master mix of reagents (no template). Prepare, mix well, and aliquot desired volume appropriately. This will minimize pipetting errors. Add template to each final reaction mix as the final step.

e.Gently mix all components, and then centrifuge briefly to collect the solution at the bottom of the tube or wells.

Component	20 µL reaction system	Final concentration
5×AK Taq One Step RT-PCR Buffer	4 µL	1×
AK Taq One-Step Enzyme Mix With UDG (Plus)	11 µL	Optimized enzyme units
Primer mix*	0.5~2.5 µL	0.2~1.0 µM
RNA Template	Variable	Variable
ddH2O	Up to 20 µL	—
Total volume	20 µL	—

* Primers with Tm values above 55℃ are recommended. The

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recommended final primer concentration is 0.2 μM; fine-tune primer concentration when needed.

【PCR conditions】

Transfer reactions to a qPCR machine for thermocycling using the rapid cycling conditions provided in the table below:

Step Name	Temperature	Time	Cycles
Reverse transcribing	50℃	2-5 min	1
Heating	95℃	2-5 s	1
Denaturing	95℃	1-3 s	40~45
Annealing and stretching	Tm of primer	13-20 s	

Note：Adjust cycling parameters accordingly if reaction volume is not 20 μl

【Experimental example】

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【Relative products】

MDAD035、MDE013-6

【Date of approved revision of the specification】

April 01, 2024

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