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Digital PCR One-Step RT advanced Kit for probes

Product Number: DDP0005-24T

Shipping and Storage

Store the kit at -20±5℃ with a shelf life of 12 months.

After thawing, the general master mix can be stored at 2~8°C for 1 week. Do not freeze and thaw repeatedly more than 3 times.

Components

Component	24T
One-Step Reverse Transcription Droplet Digital PCR Master Mix	250μL
Reverse Transcriptase	12.5μL

Note: Components from different batch numbers cannot be mixed.

Description

Droplet digital PCR (ddPCR) enables absolute quantification without relying on standard curves or reference samples, and directly detects the copy number of target sequences. The principle is to partition a standard PCR reaction into numerous tiny reactors. Each reactor contains zero or multiple copies of target RNA templates, realizing single-molecule template reverse transcription and PCR amplification. After amplification, a biochip analyzer detects the fluorescence intensity of each droplet individually. Compared with negative droplets, positive droplets containing nucleic acids show enhanced fluorescence signals. Finally, the absolute copy number of target nucleic acids is calculated based on the proportion of positive droplets in accordance with the Poisson distribution.

This master mix contains DNA polymerase, dNTPs, magnesium chloride and buffer. Users only need to add appropriate primers, probes, reverse transcriptase, templates and nuclease-free water to perform one-step reverse transcription droplet digital PCR. The operation is simple and convenient.

Application

Mix this master mix with self-prepared primers and probes, and use it together with the droplet digital PCR system for pre-treatment prior to droplet generation.

Required Consumables & Equipment (to be prepared by users)

1. Consumables: General sample preparation consumables, droplet generation oil, pierceable heat-sealing film for 96-well plates, 96-well PCR plate, microplate sealer (Model S100), pipettes, thermal cyclers, nuclease-free centrifuge tubes, nuclease-free filter pipette tips.
2. Nuclease-free water

Sample Requirements

Test samples are total RNA extracted from whole blood, plasma, serum, FFPE tissues, frozen tissues, fresh tissues and cells using conventional RNA extraction methods.

The recommended total RNA input per reaction is 10ng ~ 500ng. RNA amounts below 10ng or above 500ng may affect droplet generation and detection accuracy.

Protocol

1. Reaction System Preparation

1.1. Thaw all components to room temperature. Fully dissolve, mix by vortexing and perform a brief centrifugation.

1.2. Prepare the reaction mixture at room temperature according to Table 2. Combine all components except samples first, mix thoroughly and aliquot into reaction tubes. Add samples last.

Components	Volume	Final Concentration
One-Step Reverse Transcription Droplet Digital PCR Master Mix	10μL	1 ×
Target Gene Primers & Probe (FAM)	XμL	Recommended: Primers 900nM / Probe 250nM
Reference Gene Primers & Probe (VIC/HEX)	XμL	Recommended: Primers 900nM / Probe 250nM
Reverse Transcriptase	0.5μL	200U/μL
Sample	Appropriate volume	-
Nuclease-free Water	Appropriate volume	-
Total Volume	20μL	-

1.3. Vortex the PCR mixture and centrifuge briefly to collect all liquid at the bottom of tubes. Let the tubes stand at room temperature for 3 minutes.

2. Droplet Generation

2.1. Place the droplet generation chip into the chip holder. Add 50μL droplet generation oil to the oil well, and 20μL prepared PCR mixture (containing template) to the sample well. Then add 5μL sealing agent on top of the aqueous phase of each sample well. After loading oil and aqueous solutions, attach the sealing gasket and place the chip into the sample preparation instrument for droplet generation.

2.2. After droplet generation, carefully transfer the produced droplets (approximately 45~55μL) into a PCR plate one by one.

2.3. Seal the plate with pierceable heat-sealing film using a microplate sealer (setting: 190℃, 5sec). Perform PCR amplification within 30 minutes after sealing, or place the plate at 4℃ and complete amplification within 4 hours.

3. PCR Amplification

Place the sealed PCR plate into a thermal cycler. The recommended program is shown below:

Step	Temperature	Duration	Cycles	Ramp Rate
Step 1	55℃	30min	1	1℃/sec
Step 2	95℃	10min	1	1℃/sec
Step 3	95℃	30sec	45	1℃/sec
	60℃	1min	1	1℃/sec
Step 4	98℃	10min	200U/μL	1℃/sec
Step 5	Hold at 16℃			

Note: The annealing temperature can be adjusted according to the optimal Tm value of primers; Set the hot lid temperature to 105℃ and sample volume to 50μL; Any self-modified PCR program shall be verified by users.

4. Droplet Detection & Result Analysis

Load the PCR plate onto the MicroDrop-100B Biochip Analyzer for detection. Refer to the user manual of MicroDrop-100B Biochip Analyzer for detailed operation and data analysis.

Interpretation of Test Results

This one-step reverse transcription ddPCR master mix is a universal reagent for ddPCR systems. It can be used with specific fluorescence probe-based PCR detection kits for gene mutation analysis, copy number variation (CNV) analysis and absolute quantification of target genes. Please refer to the corresponding product manuals for result interpretation.

Limitations of the Test

Test results are affected by sample collection, handling, transportation and storage. Improper operations in any link may cause false negative results. Cross-contamination during sample processing may lead to false positive results.

Note

1. This kit is for research use only. Please read the manual carefully before use.
2. Thaw all reagents to room temperature before use. Mix gently by inverting tubes after full dissolution, avoid foaming, and centrifuge briefly prior to use.
3. Avoid repeated freeze-thaw cycles, which may degrade reagent performance. For long-term storage, keep the master mix at -20±5℃. For frequent short-term use, store at 2~8℃.
4. Test results may be influenced by sample source, collection, quality, transportation, pre-treatment, RNA extraction quality, operating environment and inherent limitations of molecular biology techniques, which may cause false positive or false negative results. Users shall acknowledge potential errors and limitations in accuracy during testing.
5. Be fully familiar with the operation and precautions of all relevant instruments before experiments.
6. All chemical reagents carry potential hazards. This kit shall only be operated by personnel with valid PCR laboratory certificates. On-site training by our technical staff is required for first-time users. Wear standard lab coats and disposable gloves during operation. In case of accidental splashing into eyes, immediately flush eyes with an eyewash station or plenty of clean water.
7. All test samples and kit controls are regarded as infectious materials. Sample handling and waste disposal must comply with the General Biosafety Guidelines for Microbiological and Biomedical Laboratories and Regulations on the Administration of Medical Wastes issued by national health authorities.
8. Clinical laboratories shall strictly follow the Administrative Measures for Clinical Gene Amplification Laboratories in Medical Institutions and other relevant regulatory specifications.