Real Time PCR Kit for Aspergillus fumigatus/Aspergillus flavus/Aspergillus terreus/Aspergillus niger DNA（Real-Time PCR Method）

Instruction Manual

REF **KRT02308**

Store the kit and its components at -20ºC

For Research Use Only

RUO

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Background

The Real Time PCR Kit for Aspergillus fumigatus/Aspergillus flavus/Aspergillus terreus/Aspergillus niger DNA（Real-Time PCR Method） is a molecular assay developed for detection of Aspergillus fumigatus/Aspergillus flavus/Aspergillus terreus/Aspergillus niger DNA(Real-Time PCR Method) DNA using real-time PCR technology. The kit is suitable for molecular pathogen detection and laboratory research where reliable nucleic acid detection is required from validated clinical, environmental, food, vector or research specimens, as applicable to the target organism and intended use. It offers a streamlined workflow for molecular diagnostic support, surveillance, outbreak investigation, pathogen confirmation, viral or microbial load assessment and laboratory research. The assay should be performed by trained personnel in appropriately equipped molecular laboratories. Results should be interpreted together with clinical presentation, specimen type, sampling quality and other laboratory findings. Sample types, analytical performance and regulatory claims should be validated according to local requirements before routine use.

Intended Use

A real-time PCR kit for rapid, sensitive and specific detection of Aspergillus fumigatus/Aspergillus flavus/Aspergillus terreus/Aspergillus niger DNA(Real-Time PCR Method) DNA in validated specimens for molecular diagnostic support and laboratory research.

Principle of PCR Detection

The kit uses TaqMan probe real-time fluorescence PCR technology with a pair of primers specific to the conserved region of the target analyte. A specific probe is used to amplify and detect the DNA of the conserved region of target analyte.

Kit Components

1. PCR Master Mix (Lyophilized) - 1 vial
2. Exogenous Internal Control (Lyophilized) - 1 vial
3. Positive Control (Lyophilized) -1 vial
4. Negative Control – 1 ml
5. Diluent – 1.5 ml
6. Instruction Manual

Additional Requirements

Real-time fluorescence PCR instrument with 2/3 channels.

Stability And Storage

The kit should be stored at -30ºC in dark away from direct light. It is recommended to aliquot the reagents and store to avoid repeated freeze-thaw. The number of repeated freezing and thawing should not exceed 3 times.

Application Scope

Intended for the qualitative and/or quantitative detection of target analyte in clinical specimens using real-time PCR technology.

Features

1) Lyophilized reagents offer ease of use, handling and storage
2) Reaction Volume is 25ul
3) Exogenous Internal Control provided
4) Separate Positive and Negative Control provided in the kit

Format

Ready-to-use

General Precautions

The user should always pay attention to the following:

- Use sterile pipette tips with aerosol barriers and use a new tip for every procedure.
- Store all extracted positive material (specimens, controls and amplicons) away from all other reagents and add it to the reaction mix in a distantly separated facility.
- Thaw all components thoroughly at room temperature before starting an assay.
- When thawed, mix the components and centrifuge briefly.
- Use disposable protective gloves and laboratory cloths, and protect eyes while samples and reagents handling. Thoroughly wash hands afterwards.
- Do not eat, drink, smoke, apply cosmetics, or handle contact lenses in laboratory work areas.
- Do not use a kit after its expiration date.
- Dispose of all specimens and unused reagents in accordance with local regulations.
- Samples should be considered potentially infectious and handled in biological cabinet in compliance with appropriate biosafety practices.
- Clean and disinfect all samples or reagents spills using a disinfectant, such as 0.5 % sodium hypochlorite or another suitable disinfectant.
- Avoid samples and reagents contact with the skin, eyes, and mucous membranes. If these solutions come into contact, rinse the injured area immediately with water and seek medical advice immediately.
- Safety Data Sheets (SDS) is available on request.
- Use of this product should be limited to personnel trained in RNA amplification techniques.
- Workflow in the laboratory must be one-directional, beginning in the Extraction Area and moving to the Amplification and Detection Area. Do not return samples, equipment and reagents in the area where the previous step was performed.

Sampling and Handling

Applicable sample type:
Whole blood, plasma, serum, cerebrospinal fluid, vesicle/swab specimens, tissue samples, or other validated clinical specimens, depending on laboratory validation and regulatory requirements.

Tissues Samples:
Weigh about 1 g of sample from 3 different locations of each tissue, chop it with surgical scissors and mix it, then take 0.5 g and grind it in a grinder, add 1.5 mL of physiological saline and continue grinding. After homogenization, transfer it to a 1.5 mL sterile centrifuge tube, centrifuge it at 8000 rpm for 2 min, and take 100 ul of the supernatant in a 1.5 mL sterile centrifuge tube.

Sample storage and transportation:
The collection or processing sample should not exceed 24 hours under the conditions of 2°C ~ 8°C. If long-term preservation is needed, it should be stored below -70°C, and the freezing fusion should not exceed 3 times.

Reagent Preparation

1) Take out the PCR Master Mix, open the bottle cap according to the arrow direction of the aluminum-plastic cover, add 1 mL of Diluent, strongly mixed on the vortex for more than 1 minute, then stand for 30 ~ 60 seconds until the liquid is clear and transparent. Pipette it into PCR reaction tubes according to 19 uL / tube.
2) Take out the Exogenous Internal Control and open the tube cap, add 100 uL of Diluent, then cover the tube, mix evenly for 15 seconds with a vortex shaker, centrifuge for 3 seconds with a palm centrifuge, and then Pipette it into PCR reaction tubes according to 1 uL / tube.

The total test reaction system mixed well between the PCR Master Mix and Exogenous Internal Control is 20 ul / tube.

Assay Protocol

A) Nucleic Acid Extraction

- This kit is not included with Nucleic Acid (NA) extraction reagent.
- We recommend using commercially available extraction kits that have been shown to generate highly purified DNA.
- If the extracted DNA is not used immediately, it should be stored below -20ºC.
- For long-term storage, it should be stored below -80℃ and avoid repeated freezing and thawing.

Note: The Negative Control and the Positive Control do not require nucleic acid extraction to be done.

B) Addition of Sample

Samples are to be added to the above prepared PCR reaction tubes according to the following table:

Sample /Control	Protocol
Sample	Add 5 ul of the extract prepared to the reaction tube, and cover the tube.
Negative Control	Add 5 ul of negative control to the reaction tube, and cover the tube.
Positive Control	Add 5 ul of positive control to the reaction tube, and cover the tube.

The total reaction volume is 25 ul

After adding the sample, the PCR reaction tubes should be centrifuged for 15 second on a palm centrifuge. If bubbles are found, the tube wall should be gently flicked to remove bubbles and centrifuged again.

C) Amplification

Place the reaction vial in the automatic fluorescent PCR instrument.
Set the Negative Control, Positive Control, and test Sample template parameters to perform PCR experiment according to the operating instructions of the instrument, and record the corresponding sample name.

Select FAM channel to detect template, select VIC channel to detect Exogenous Internal Control. Set the Reaction Volume per Well to 25 ul.

(Note: For ABI series instruments, select 'None' under 'Quencher' , and select 'None' as the dye to use as the passive reference.)

Create a temperature profile on the RT PCR instrument as follows:

Step	Cycle	Temperature	Time	Collect Fluorescence Signal
1	1	95ºC	2 min	No
2	45	95ºC	15 s	No
		60ºC	30 s	Yes

Data Analysis

After the reaction is completed, the results are automatically saved.

- The Start Value, End Value and Threshold Value of the Baseline should be adjusted according to the analysed image (the user can adjust it according to the actual situation, the Start Value can be set at 3~15, the End Value can be set at 5~20, the amplification curve of the Negative Control should be adjusted to be flat or below the threshold line) .
- Click Analyse for analysis and then go to the Plate window to record the Ct Value.

Quality Control
The test is valid if the following conditions are met -

Negative Control:
FAM detection channel has no obvious amplification curve.

Positive Control:
FAM detection channel has an obvious amplification curve, and the Ct value ≤ 32.00.

Exogenous Internal Control:
VIC detection channel has an obvious amplification curve, and the Ct value ≤ 32.00.

The above requirements must be met at the same time in the same experiment; otherwise this experiment is invalid and needs to be repeated.

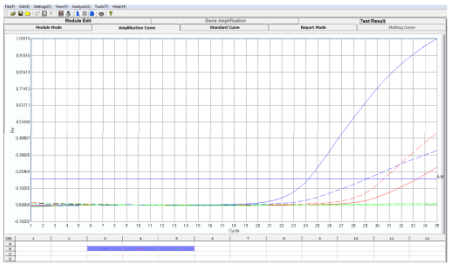
Interpretation of Results
The FAM channel is the detection result of the target analyte, and the VIC channel is the detection result of the Internal Control.

Positive:
Ct value ≤40.00 and the curve has a clear index growth curve.

Negative:
Ct value >40.00 or no Ct value.

Suspicious samples: If the sample test result is 40.00 < Ct value ≤ 45.00, it is recommended to repeat the test. If the test channel is still 40.00 < Ct value ≤ 45.00 and the curve has an obvious growth curve, it is judged as positive, otherwise it is negative.

The result of the analysis is considered reliable only if the results obtained for Positive and Negative Controls of amplification as well as for the Negative Control of extraction are correct.



Typical Amplification Curve of the RT PCR reaction with a clear index growth curve

Performance Characteristics

Limit of Detection:
The minimum detection limit of this reagent is 5 copies / reaction.

Precision:
Repeat detection of the enterprise precision reference product 10 times, and the Coefficient of Variation (CV, %) of the Ct value of the target detection channel is ≤ 5.00%.

Compliance rate of negative/positive reference products:
The compliance rate of negative samples is 100%, and the compliance rate of positive samples is 100%

Sensitivity: 100%
Specificity: 100%

Troubleshooting

Please contact the manufacturer in case of a problem during a run.

Typical Causes:
Signal from FAM/Cy5 Filter in the Negative Control
Probable Cause: Contamination
Action Suggested: Use filter-tips. Repeat PCR with new kit components.

The Threshold is Above Low Signals
Probable Cause: The threshold should be manually adjusted
Action Suggested: Using the mouse pull the threshold down until it cuts the low signals. Avoid the background and the signal from negative control.

Symbols


Use by

Lot/Batch

Catalog number


Temperature limitation


Caution, consult accompanying documents


Manufacturer

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