Mucorales PCR Detection Kit (Lyophilized)
User Manual

【PRODUCT NAME】
Mucorales PCR Detection Kit (Lyophilized)

【SIZE】
48tests/kit, 48tests/kit, 50tests/kit

【INTENDED USE】

Mucormycosis is a serious but rare fungal infection caused by Mucorales， which live throughout the environment. Mucormycosis mainly affects people who have health problems. Mucorales can also infect people with normal immunity who underwent subcutaneous traumatic inoculation. Invasive mucormycosis can result in rhino-orbitalcerebral, pulmonary, gastrointestinal, cutaneous, widely disseminated, and miscellaneous infection. In many cases, the disease progresses rapidly and may result in death unless underlying risk factors are corrected and appropriate antifungal therapy and surgical excision are initiated.

This kit is intended to *in vitro* qualitatively detect the 18S ribosomal DNA gene of Mucorales in the bronchoalveolar lavage (BAL) and Serum samples samples collected from cases and clustered cases suspected with Mucormycosis.

【PRINCIPLE OF DETECTION】

This product is a fluorescent probe-based Taqman PCR assay system. PCR amplification will be performed with the DNA of Mucorales as template. During amplification of the template, the Taqman probe will be degraded due to the 5'-3' polymerase activity and exonuclease activity of Taq DNA polymerase, then the separation of fluorescent reporter and quencher enables the fluorescent signal to be detected by instrument. The 18S ribosomal DNA gene of Mucorales will be detected qualitatively by FAM channel, and the human internal reference gene will be detected by **ROX** channel. Internal reference is used in the kit for quality control starting from sample collection to avoid false negative results..

【PRODUCT CONTENTS】

Components	Package	specification	Ingredient
Mucor PCR Mix	1 *bottle (Lyophilized)	50Test	dNTPs, $MgCl_2$, Primers , Probes, Taq DNA polymerase
	6*0.2ml 8 well-strip tube (Lyophilized)	48Test	
	3*0.2ml 8 well-strip tube (Lyophilized)	48Test	
Positive Control	2*0.2ml tube (lyophilized)	8Tests	Plasmid containing 18S ribosomal DNA gene, and IC gene specific fragments
Dissolving solution	1.5 ml Cryotube	800uL	/
Negative Control	1.5 ml Cryotube	200uL	0.9%NaCl

Note: Do not mix the components from different batches for detection. The positive control of Mucor and internal reference were constructed artificially, and they were not infectious.

【STORAGE & SHELF LIFE】

(1) The kit can be transported by Normal transport.
(2) All kit components can be stored at 2℃~30℃ with protection from light. And the kit is stable for 12 months when stored at the recommended condition.
(3) See label of outside box for production date and expiration date.
(4) The lyophilized reagent should be stored at -20℃ after dissolution and the repeated freeze -thaw should be less than 4 times.

【INSTRUMENTS】

Our recommendation for platform to use Mucor PCR Detection Kit: Real-time PCR instrument-- Roche LightCycler 480、Life Technologies 7500、Molarray MA-688, Analytikjena qTOWER serials, and other real-time fluorescence PCR instruments with FAM, ROX channels.

【SAMPLING & HANDLING】

(1) Serum: Collect Serum sample for test.

(2) Bronchoalveolar Lavage: Collect bronchoalveolar lavage correctly for test.

The collected sample should be used for detection as soon as possible. If the sample need to be transferred cannot be detected immediately, please store it at low temperature.

The sample can be stored for 24 hours at 2~8℃ and for a long time below -70℃. It can also be stored in refrigerator at -20℃temporarily.

Samples shall be transported at low temperature in accordance with biosafety regulations.

【PROTOCOL】

1. Reagent Preparation

a) Lyophilized powder version kit:Add 750uL dissolving solution to the bottle to dissolve the lyophilized powder. Divide 15uL of the dissolved reagent into each PCR reaction tube.

b)Lyophilized 8-well strip version kit: Add 15uL dissolving solution to each tube well to resolve the PCR Mix.

c)Positive Control :Add 20uL dissolving solution to one tube well of positive control to resolve it.

Shock and centrifuge them at low speed. The dissolved reagent and positive control can be temporarily stored at 4℃ for later use.

*Notes:When using the lyophilized in bottle version kit, after dissolving the reagent can be stored at -20℃ and repeated freeze -thaw should be less than 4 times.

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2. DNA Extraction

Extract the nucleic acid(DNA) from the specimen using appropriate nucleic acid extraction kit and following the instructions of extraction kit.

After DNA extraction, the extracted DNA shall be added to the PCR reaction tubes within 15 minutes, or transferred to the centrifuge tubes and stored at -15 ℃~-25 ℃.

3. Template Addition

Add 5 μL Negative Control, 5 μL Positive Control, and Add 5 u L extracted nucleic acid of each specimen into each PCR reaction tube. Shock and centrifuge them at low speed. Then, move them to the Real-time PCR instrument.

4. PCR Amplification

Recommended Setting

	Step	Temperature (℃)	Time	Cycle
1	Pre-denaturation	95	2mins	1
2	Denaturation PCR	95	10s	40
3	annealing/extension	60	20s	

*Note: The signals of FAM, ROX fluorescence channels will be collected at 60℃. Select "None" for the passive reference on operation interface of ABI7500 PCR software.

5. Data Analysis (ABI7500)

Test data file need to be saved after PCR reaction. Please set the parameters and analysis the results of FAM, ROX channels respectively.

(1) Baseline setting: the baseline can be set automatically or adjusted according to the shape of amplification curve.

(2) Threshold setting: the threshold value should be higher than the highest fluorescence value of negative control in this kit.

6. Quality Control

Negative control and positive control provide the quality control for the assay and shall be conducted for each run of test. The result is valid if ALL the below criteria is met. Otherwise, the test is invalid. In this case, the errors of instruments, reagents, amplification conditions, etc. shall be checked, and the experiment shall be repeated.

Products of Quality Control	Requirements of Quality Control	
	FAM Channel	ROX Channel
Positive Control of Mucor	Ct ≤ 32	Ct ≤ 32
Negative Control	Undet	Undet or Ct>38

7. Interpreting Test Results

Channel		Interpretation of results
FAM Channel	ROX Channel	
Ct≤38	Ct≤32	Mucor Positive
Ct>38	Ct≤32	retest*
Undet	Undet or Ct>32	re-sampling and retest

*If any retest result of FAM channels has a Ct value >38, the result is interpreted as positive, otherwise it is negative. If the result of the internal control(ROX channel) Ct is Undet or Ct＞32 the test result is invalid and re-sampling and retest should be done.

【CUT-OFF VALUE OR REFERENCE INTERVAL】

The cut-off value of Mucor is Ct ≤ 38.

【ASSAY EXPLAINATION】

1. The contamination of laboratory environment and reagent, or cross contamination during specimen treatment may lead to false positive result.

2. The decrease of detection effect even the false negative result may occur if there is any mistakes in the transportation, storage and operation of reagents.

3. Mucor early infection or other respiratory virus infection can't be excluded in patients with negative results. If conditions permit, it is recommended to collect more sensitive samples such as sputum or bronchoalveolar lavage for retest.

【ASSAY LIMITATIONS】

1. The positive result detected by this kit can't indicate whether there is virus in vivo. It is suggested to use other methods for confirmation at the same time.

2. This kit is intended for classification and detection of Mucor. The result is only for clinical reference, and the clinical management of patients should be considered in combination with their symptoms/signs, history, other laboratory tests and treatment responses.

3. Although the detected target sequences of this kit are the conservative region of Mucor's gene, the missed detection of coronavirus types with rare mutations in the conservative region can't be completely avoided in theory.

【PERFORMANCE SPECIFICATIONS】

Conformity rate of Negative Control: detection results of Mucor were negative in 8 enterprise reference samples (T1-T8), and the conformity rate of negative control (-/-) should be 8/8.

Conformity rate of Positive Control: detection results of Mucor were positive in 4 enterprise reference samples (Y1-Y5).

Detection limitation: 1000 copies /mL or 5copies/ Reaction

Repeatability: The test results of enterprise reference samples (J1) were all positive after 10 repetitions, and the coefficient of variation (CV) of J1's Ct value is less than 5.0%.

Precision: 5 days of continuous testing, 2 times a day for each person, 4 repetitions for each sample, and the coefficient of variation (CV) of their Ct value is less than 5.0%.

Specificity: non-specific interference of other related pathogens (Coronavirus (229E, HKU1, OC43, NL63), Influenza A Virus (H1N1, H3N2), Influenza B Virus, Canine coronavirus, Avian influenza H7N9.

【ATTENTIONS】

1. The kit is only used for in vitro diagnosis.
2. Please read this manual carefully before beginning the experiment.
3. All equipment used in the experiment shall be sterilized.
4. Unreasonable sample collection, transfer, storage and operation may lead to wrong test results.
5. DNA extraction shall be carried out as soon as possible after sample collection to avoid degradation. If it cannot be carried out immediately, it shall be stored in accordance with **[SAMPLING & HANDING]**.
6. After the operation of the nucleic acid extractor, the used consumables shall be sealed. After the instrument is cleaned, turn on the ultraviolet lamp for 30 minutes.
7. As this test involves the extraction of DNA and PCR amplification, please take care to avoid contamination of the amplification reaction mixture. Regular monitoring of laboratory contamination is recommended.
8. When using this kit, please strictly follow the instructions. The collection, storage and transfer of samples, the extraction and detection of DNA, and the interpretation of results must be carried out in strict accordance with the requirements of the kit instructions. The processes of sample preparation and addition must be carried out in the biosafety cabinet or other basic protective facilities according to the technical requirements of the clinical gene amplification laboratory.
9. Mucor has strong transmission ability and high-risk coefficient. Personal protection should be a three-level laboratory level of biosafety. The operator must have professional skills and PCR inspection qualification.

During the whole operation process, it is necessary to prevent the infection risk of aerosol pollution, and the operator must add samples and use reagents and consumables accurately.

10. To prevent virus spreading, the Mucor must be detected in a biosafety level 2 (P2) or above laboratory. Laboratory management should strictly follow the management standard of PCR gene amplification laboratory, and the experimental operation must be strictly partitioned. The instruments, equipment, consumables, work clothes used in each region must be distinguished strictly and can't be used intercross to avoid contamination.

11. All test samples shall be regarded as infectious substances. During the experiment, work clothes shall be worn, disposable gloves shall be worn and replaced frequently to avoid cross contamination between samples. The operation of sample and waste shall meet the requirements of relevant laws and regulations.

【Literature References】

[1] Mercier, T.; Reynders, M.; Beuselinck, K.; Guldentops, E.; Maertens, J.; Lagrou, K. Serial Detection of Circulating Mucorales DNA in Invasive Mucormycosis: A Retrospective Multicenter Evaluation. J. Fungi 2019, 5, 113. https://doi.org/10.3390/jof5040113.

【General Information】

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