

Pet Pathogen Nucleic Acid Detection Kit

USER MANUAL

[Packaging]

PCR amplification reagent 8 tests
User manual 1 piece

[Intended Use]

This kit uses Fluorescence-based PCR analysis to detect pathogens in pleural effusion, ascites, stool, eye/nasopharyngeal swab, and blood specimens from canine, feline or other animals. It is applicable to the detection, diagnosis and epidemiological investigation of pet pathogens.

[Methodology]

Use nucleic acid extraction kits to extract nucleic acids (DNA/RNA) from specimens. Under the action of highly efficient reverse transcriptase, cDNA strands complementary to RNAs are synthesized during the 1-step reaction, serving as DNA templates. When Taq DNA polymerase functions, copies of specific target sequences are amplified after cycles of denaturation, annealing and extension. Fluorescent probes hybridize to the amplifying target DNAs. The 5'→3' exonuclease activity of Taq DNA polymerase then causes the reporter and quencher groups of probes separate from each other, generating specific fluorescence. The fluorescent PCR instrument detects fluorescent signals, and analyzes the results based on the Ct values and the amplification curves.

[Storage and shelf life]

Storage temperature 4°C-30°C; shelf life: 18 months, please use it within the expiration date.

[Operating environment]

Recommended operating temperature is 20-25 °C.

[Specimens]

Pleural effusion, ascites, stool swabs, anal swabs, eye/nasopharyngeal swabs, EDTA anticoagulant blood.

[Sampling requirements]

Liquid sample: (including blood sample, abdominal effusion, pleural effusion or other liquid samples, etc.)
Directly take 200µL and add it to LB well containing lysis buffer for lysis. If the sample is viscous, dilute it with preservation buffer, and then add 200µL solution to LB well for lysis.

Swab sample:

1. Fecal swab: take proper amount with a swab
2. Anal swab: moisten the swab with dilution buffer, and then wipe specimen
3. Eye/nasopharyngeal swab: swab under the eyelid and fully wipe to collect eye swab samples; swab oral and nasal discharge properly to collect nasopharyngeal swab samples
4. After swab sample collection, quickly snap the swab handle and place in the preservation buffer, and then shake it to fully dissolve the pathogen on the swab into the preservation buffer.

It is recommended that nucleic acid extraction and detection should be performed immediately after sample collection. If storage needed, samples can be stored at 4°C temporarily or below -20°C for a longer time. Samples need to be refrigerated during transportation.

[Preparation before testing]

1. Please read the instruction manual carefully before testing, be familiar with each step, and strictly follow the requirements when using the kit.
2. Wear disposable gloves and masks, operate under the condition meeting the requirement of PCR testing environment.

Before PCR amplification, nucleic acid extraction must be completed by extraction reagent

PCR amplification: (using real time PCR analyzer)

1. Use a pipette to draw 20µL of nucleic acid solution from the EB well of the cartridge and add it to the PCR testing tube. Flick the tube with your finger to fully dissolve and mix the lyophilized reagent. Shake all the solution in the tube to the bottom (or centrifuge for 5-10 second) and put it on the reaction well of the PCR instrument.

Note:

- a) Do not suck up the bubble in the solution when pipetting the nucleic acid solution
 - b) The lyophilized reagent must be completely dissolved and mixed, and all the reaction liquid in the tube must be shaken to the bottom. Otherwise, strong background signal interference might happen easily, which will affect the result analysis.
2. Select the corresponding program for the specific pet pathogens, set the sample and test information, and then press the operation icon to start the reaction.
 3. When the reaction is completed, take out the PCR product tube immediately. Seal it with a zip bag and dispose of it properly.

[Result interpretation]

1. When the FAM detection channel shows an S-type amplification curve, and the Ct value is < 38, the result is positive.
2. When the FAM detection channel shows no S-type amplification curve, as there is no Ct value or the Ct value is ≥38, the result is negative.

[Limitations]

1. Aerosol contamination of amplification products can easily cause false positive results. The testing laboratory should be set up, strictly following the requirements of the PCR testing laboratory
2. A negative result cannot completely exclude the possibility of pathogen infection and needs to be diagnosed with other clinical indicators.

[Product quality indicators]

1. Sensitivity: The product detection limit of the kits is 1000 copies/ml.
2. Specificity: The test kits do not detect the cross-reaction of the pathogen samples.
3. Precision: When a strong positive sample and a weak positive sample are repeated testing for 10 times in a row separately, the CV values of their Ct values show less than 5%.

[Precautions]

1. Product quality inspection: Before using PCR amplification reagent, unpack to check if the lyophilized reagent on the tube bottom is normal (white, clumped). If liquefied, it cannot be used. Otherwise, it will affect the PCR results.
2. Pipette use: When drawing 20µL of nucleic acid supernatant as the template for PCR amplification, only depress the plunger of the pipette to the first stop. Do not press to the bottom, otherwise the sample volume will exceed 20µL which will affect the results.
3. Sample information setting: Make sure that the PCR tubes are placed in the same sample well position as set in the instrument. For example, if the PCR tube is placed in the #1 well of the sample plate, then select the corresponding #1 position in the interface. After setting the sample name and the test item, press the Run icon to start the PCR amplification process.



寵物病原體

PCR 試劑

使用說明書

【試劑盒組成與規格】

PCR試劑8個
說明書 1份

【預期用途】

本試劑盒採用螢光PCR方法檢測犬、貓等動物的胸水、腹水、糞便、眼鼻咽拭子、血液等樣品中的病原體，適用於寵物病原體的檢測、診斷和流行病學調查。

【檢驗原理】

使用核酸提取試劑提取樣品的核酸(DNA/RNA)，在高效反轉錄酶的作用下，以RNA為範本，經一步反應合成與 RNA 模板互補的cDNA鏈。在Taq酶的作用下，以DNA為模板，經變性、黏合及延伸的循環，使特异性目的片段的複製放大。經螢光標記的特異性探針與擴增的目的片段雜交，利用Taq聚合酶的5'→3'外切活性，使螢光探針的螢光基團與消光基團分離，發出特異性螢光信號，利用螢光PCR儀檢測特異性螢光信號，根據樣品 Ct值的大小及擴增曲線的形成情況判定結果。

【儲存條件與有效期】

試劑盒儲存溫度：4°C-30°C
試劑盒有效期18個月，請於有效期內使用

【儀器工作環境】

建議在環境溫度20-25°C下進行實驗

【樣本類型】

胸水、腹水、糞便拭子、肛拭子、眼鼻咽拭子、EDTA抗凝全血

【採樣要求】

液體樣本：(包括血液樣品、腹腔積液、胸腔積液或其他液體樣品等)
直接取200μL加入到裂解液LB管中進行裂解，如樣品粘稠時則先用保存液稀釋，再取200μL加入到LB管中進行裂解

拭子樣本：

1. 糞便拭子：用拭子蘸取適量即可
2. 肛拭子：先把拭子用稀釋液濕潤，然後再擦拭取樣
3. 眼鼻咽拭子：用拭子在眼瞼下，充分擦拭以採集眼拭子樣品；用拭子適度拭抹口腔和鼻腔分泌物，採集鼻咽拭子樣品
4. 拭子樣品採集後，應迅速將拭子頭折斷於保存液中，然後充分震盪，使拭子頭上的病原體充分溶解到保存液中。

樣品採集後建議應馬上進行核酸萃取檢測；如需存放，可於4°C短暫保存或-20°C以下長期保存；樣品送檢運輸時，需要冷藏運輸。

【實驗前準備】

1. 請在實驗開始前仔細閱讀本說明書，熟悉各個步驟，並嚴格按照本說明書的要求使用本試劑盒
2. 操作過程應戴好一次性手套和口罩，並在符合PCR檢測實驗環境的條件下進行操作

進行PCR擴增前，需先依照核酸萃取試劑說明完成核酸萃取

PCR擴增：(使用即時螢光PCR儀)

1. 使用微量分注器從萃取盒的EB孔中取20μL核酸溶液，加入到PCR試劑管中，用手指彈動試劑管使乾粉充分溶解混勻，然後把管內所有液體甩至管底(或使用離心機離心5-10秒)，再放入PCR儀反應孔位上

注意：

- a) 用微量分注器吸取核酸溶液時不要吸取到溶液中的氣泡
 - b) 凍乾試劑一定要充分混勻，且一定要將管內所有反應液都甩至管底，否則易造成背景干擾信號過強，從而影響結果判斷。
2. 選擇寵物病原體相應程式，設置樣本和檢測專案資訊，點擊運行圖示，開始反應
 3. 反應結束後，及時取出PCR產物試管，並用夾鏈袋封裝後統一處置。

【結果判讀】

1. 當FAM檢測通道有典型S型擴增曲線，且Ct值<38時，結果判斷為陽性。
2. 當FAM檢測通道無典型S型擴增曲線，即無Ct值或Ct值≥38時，結果判斷為陰性。

【檢測方法的局限性】

1. 擴增產物的氣溶膠污染很容易造成假陽性，檢測實驗室應嚴格按照PCR檢測實驗室的要求設置。
2. 陰性結果不能完全排除病原體感染的可能，需結合其他臨床指標進行判斷。

【產品性能指標】

1. 靈敏性：本試劑盒的產品檢測限為1000 copies/ml。
2. 特異性：本試劑盒非檢測病原體樣本無交叉反應。
3. 精密度：一份強陽性和一份弱陽性的標本分別連續重複10次檢測，其Ct值的CV值小於5%。

【注意事項】

1. 產品檢查：使用PCR擴增試劑前，先拆開包裝檢查管底乾粉是否正常(白色、成團)，如已經液化則不能再使用，否則會影響PCR結果。
2. 微量分注器使用：吸取20μL核酸上清作為PCR的擴增範本時，微量分注器應只壓至一檔，不能壓到最底部，否則吸取體積將超過20μL，影響檢測結果。
3. 樣本資訊設置：確保PCR管放置的孔位與儀器中設置的樣本位置一樣，如PCR管放置在擴增室中的1號位置，則在樣本設置介面選擇對應的1號進行設置，設置好樣本名和檢測項目後，點擊運行圖示即開始PCR擴增反應。

