Turkey Rhinotracheitis Real-Time Detection Kit

TRT Real-Time Detection Kit

Limit of application

TRT Real-Time PCR Kit from Animal Genetics, Inc. is useful for detecting the infection of Turkey Rhinotracheitis virus. The kit can be exactly performed to detect Turkey Rhinotracheitis virus of RNA, so it can be used for both qualitative analysis and quantitative analysis. It works most of Real-Tme PCR apparatuses of block and capillary type.

Material provided (96Tests/Kit)

Cat No. PD 65-10

No.	Product	Amount
1	Standard 1 ($1x10^4$ copies/㎕)	200㎕
2	Standard 2 ($1x10^5$ copies/㎕)	200㎕
3	Standard 3 ($1x10^6$ copies/㎕)	200㎕
4	Standard 4 ($1x10^7$ copies/㎕)	200㎕
5	Detection Solution	800㎕
6	Nuclease free Water	500㎕
7	PCR enzyme mix	100㎕
8	RT mix	100㎕

* The kits is provided extra ROX reference dye for ABI prism series (7000/7700/ 7900HT) and ABI 7300 apparatus.

Precautions

1) For veterinary research use only.
2) Remove the possibility of contamination of Nucleic acid and PCR product when processing PCR.
   It is recommended to work at the clean bench related with PCR.
3) Use sterilized Filter tip.
4) Do not make any bubbles from the test bottom when testing.
5) Detection Solution minimizes the exposure of light.
6) Do not use the kits expired validity.
7) Read the result as Avian Mycoplasm virus a by following clinical symptoms and autopsy even if the kits show the positive result. You are required to ask for testing at the quarantine center or any other epidemic control center when the results are doubted.
8) The detection limit of the kit is 10 copies. It may not detected less than 10 copies.

Kit storage and stability

The kit should be stored at -20℃. Under these conditions reagents are stable through the expiration date printed on the label.

Procedure of the test

1) RNA extraction (Template RNA)
   It is easily separated by using Rneasy kit from QIAgen (Valencia, USA, and TRIzol reagent from Invitrogen (Life Technologies, USA). Follow the manufacturer's instruction. We recommend to use TRIzol reagent for extracting high density of RNA.

2) Prepare the PCR mix.

Detection Solution (5)	8㎕
PCR enzyme mix (7)	1㎕
RT mix (8)	1㎕
Template RNA	5㎕
ROX reference dye*(optional)	0.4㎕
Adjust with Nuclease free water (6) to	20㎕

* ROX reference dye method
① Add it only when you use ABI prism series (7000/7700/7900HT) and ABI 7300 Real-Time PCR system from Perkin Elmer.
② Do not use it in ABI 7500 Real-Time PCR system or any other system. ROX reference dye for the ABI 7500 is already included in the Detection solution(5). Other Realtime apparatus doesn't required of ROX reference dye.

3) Set up the reaction samples, Standard 1, 2, 3, 4 and Negative control (Nuclease free water(6)) in separate tube.
4) Set up the Cycle Program as following the condition below.

Cycles	Reaction	Temp. (℃)	Time
1	TRTerse transcription	42℃	30 min.
1	Inactivation of RTase	94℃	15 min.
40	Denaturation	94℃	15 sec.
	Annealing / Extension	60℃	60 sec.

5)Set up the Report dye as FAM.

* Applicable Real-Time PCR apparatus
① Perkin Elmer
   ABI prism series (7000/7700/7900HT)
   ABI Real-Time PCR system (7300/7500)
② Corbett: Rotor-gene
③ BioRad: iCycler, Chromo4, Dyad Disciple cycler
④ Roche Applied Science: LightCycler
⑤ Usually this kit is available with most of general Real-time PCR apparatus.

Interpretation of the test

1) Qualitative analysis
Ct (Threshold cycle) value of each sample can be read as follows.

Ct value	result
> 40	negative
≤ 40	positive

2) Quantitative analysis
① Assess the Ct value when amplification curve of Standard 1, 2, 3, 4 passes the threshold line.
② Calculate quantitative value to compare with Ct value of unknown samples and curve of Standard 1, 2, 3, 4.
3) Test validation
① Each Ct value standard should be as follows.
Standard 1 > Standard 2 > Standard 3 > Standard 4
② R-value of standard curve should be 0.900~0.999.
③ The standard result should be all negative.

Detection Limit

1) Do not read the result when Ct value is less than 5.
2) **Anigen TRT Real-Time PCR Kit** can be detected Turkey Rhinotracheitis virus, RNA with high sensitivity, but it may be caused of false negative result due to RNA mis-extraction, operating error from unskilled researcher, denaturation of the kit, and any other unknown various reason.

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