BIONOTE Reverse Transcription PCR Kit

AniGen

One Step RT-PCR Kit®

■ Principles

The AniGenn One Step RT-PCR Kit® provides a convenient format for highly sensitive and specific RT-PCR using any RNA. The kit contains optimized components that allow both reverse transcription and PCR amplification to take place in what is commonly referred to as a "one-step" reaction.

■ Materials provided (100 Reactions/Kit)

Component	Volume
5X One Step RT-PCR Buffer(Blue cap)	1.0ml X 1vail
5X Band Clear Solution*(Yellow Cap)	1.0ml X 1vial
dNTP Mix,10mM each(Green Cap)	0.2ml X 1vial
RNase Free Water(Colorless Cap)	1.5ml X 3vials
PCR Enzyme Mix(Red Cap)	0.12ml X 1vial
RT mix(Brown Cap)	0.12ml X 1vial

* Facilitates amplification of GC-rich templates

■ Precautions

1. For research use only.
2. Perform the reaction setup in an area separate from nucleic acid preparation or PCR product analysis. It is` generally commended that the reaction setup is performed in clean bench.
3. Pipet with sterile filter tips.
4. The test tube should be force the solution to the bottom of tubes and remove any possible bubbles.
5. Do not use reagents beyond the stated expiration date marked on the package label.
6. Prepare and freeze small aliquots of primer solutions and dNTP Mix. Use of fresh distilled water is strongly recommended.
7. In case of contamination, laboratory benches, apparatus, and pipets can be decontaminated by cleaning them with 10% (v/v) commercial bleach solution. Afterwards, the benches and pipets should be rinsed with distilled water.

■Storage and Stability

This kit is shipped at +2 to +15℃. Store the kit after arrival at -20℃ or less in the dark. The test kit is stable through the expiration date marked on the package label.

■Equipment and Reagents to be Supplied by user

1. **Primers**

   The AniGen One Step RT-PCR Kit is designed to be used with **gene-specific primers**. The use of random oligomers or oligo-dT primers is not recommended.

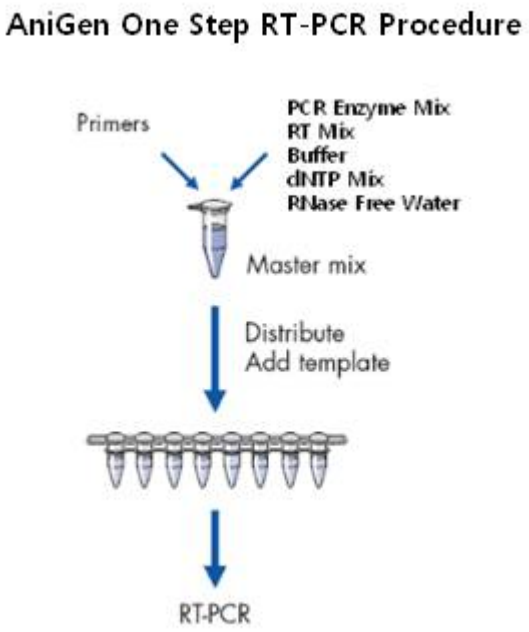
2. **RNase inhibitor***

   RNase inhibitor is a 50 kDa protein that strongly inhibits RNases A, B, (optional) and C, as well as human placental RNases. It helps to minimize the risk of RNA degradation during experimental setup.

■Important Notes before Starting

1. HotStarTaq DNA Polymerase, contained in the AniGen One Step PCR Enzyme Mix, requires initial activation by incubation at 95°C for 10 min before amplification can take place. This incubation also inactivates the reverse transcriptases. Do not heat activate the HotStarTaq DNA Polymerase until the reverse-transcriptase reaction is finished.
2. The AniGen One Step RT-PCR Kit is designed to be used with gene-specific primers at a final concentration of 0.6 µM. The use of random oligomers or oligo-dT primers is not recommended since it will result in the amplification of nonspecific products.
3. Set up all reactions on ice.
4. An RNase-free environment should be maintained during RNA isolation and reaction setup.
5. Set up the reaction mixtures in an area separate from that used for RNA preparation or PCR product analysis.

■Procedure of the test



1. Sample materials

   Use any virus template RNA suitable for RT-PCR. Template RNA can easily be prepared using kits such as RNAEasy from Qiagen (Valencia, CA, USA) and Triazol from Life Technologies (Invitron, USA).

2. To get high & pure concentrated template RNA, it is strongly recommended that the viral template RNA is extracted from a specimen by using Trizol reagent.
3. Thaw template RNA, primer solutions, dNTP Mix, 5x One Step RT-PCR Buffer, and RNase-free water, and place them on ice.
4. Prepare a master mix by serially dispensing components to each tube according to **Table1.**
5. Mix the master mix thoroughly, and dispense appropriate volumes into PCR tubes.
6. Add template RNA (≤2 µg/reaction) to the individual PCR tubes.
7. When using a thermal cycler with a heated lid, do not use mineral oil. Proceed directly to step 6. Otherwise, overlay with approximately 50 µl mineral oil.
8. Program the thermal cycler according to the program outlined in **Table 2.**

Table 1. Master Mix for One Step RT-PCR

Reagents	Volume per reaction
RNase Free Water	Variable
5X One Step RT-PCR Buffer	5μl
dNTP Mix,10mM each	1μl
Primer 1	Variable
Primer 2	Variable
PCR Enzyme Mix	0.8μl
RT mix	0.5μl
RNase inhibitor*	(Variable)
5X Band Clear Solution$	(Variable)
Template RNA	5
Total volume	25μl

*Optional : The use of RNase inhibitor is optional; because the buffer composition has an inhibitory effect on RNases.

$ Optional: 5X Band Clear Solution changes the melting behavior of nucleic acids and can be used for RT-PCR systems that do not work well under standard conditions. When using 5X Band Clear Solution the first time with a particular primer–template system, **always perform parallel reactions with and without 5X Band Clear Solution.**

Table 2. Thermal Cycler Condition

Cycles	Reaction	Temp.(℃)	Time
1	Reverse transcription reaction	42℃	30 min.
1	Inactivation of RTase	94℃	10 min.
30~40	Denaturation	94℃	0.5~1min.
	Annealing	50~60℃	0.5~1min
	Extension	72℃	1min/kb
1	Final extension	72℃	5~10 min.

9. Start the RT-PCR program while PCR tubes are still on ice. Wait until the thermal cycler has reached 42°C. Then place the PCR tubes in the thermal cycler.

■ Troubleshooting Guide

Little or no product	Comments and suggestions
Pipetting error or missing reagent	Check the concentrations and storage conditions of reagents, including primers and dNTP Mix. Repeat the RT-PCR.
HotStarTaq DNA Polymerase not activated	Ensure that the cycling program included the HotStart Taq DNA Polymerase activation step (10 min at 95°C).
HotStarTaq DNA Polymerase activated too early	Check the cycling program. Ensure that the reverse-transcription reaction is complete (30 min at 42°C) before activating the Hot Start Taq DNA Polymerase (10 min at 95°C).
Reverse-transcription reaction temperature incorrect	A reverse-transcription reaction temperature of 50°C is recommended. However, ifdesired results are not obtained using 42°C,reaction temperatures of 37–50°C may be used.
Primer concentration not optimal or primers degraded	A primer concentration of 0.6 μM is strongly recommended. However, if the desired results are not obtained using this concentration, repeat the RT-PCR with different primer concentrations from 0.5–1.0 μM in 0.1 μM increments. In particular, when performing highly sensitive RT-PCR, check for possible degradation of the primers on a denaturing polyacrylamide gel*.
RT-PCR conditions not optimal	Using the same cycling conditions, repeat the RT-PCR using 5X Band Clear Solution.
Incorrect nucleotide concentration	Use 0.4 mM of each dNTP. Different nucleotide concentrations can reduce the amount of RT-PCR product.
Enzyme concentration too low	Ensure that 2 μl of AniGen One Step PCR Enzyme Mix and RT Mix per reaction was used.
Incorrect PCR annealing temperature or time	Decrease annealing temperature in 2°C steps. Annealing time should be between 30 and 60 seconds. Difficulties in determining the optimal annealing temperature can be overcome in many cases by performing touchdown PCR
Multiband Product	**Comments and suggestions**
Reactions set up at room temperature	Be sure to set up the RT-PCR on ice to avoid temperature premature cDNA synthesis.
PCR annealing temperature too low	Increase annealing temperature in increments of 2°C. Difficulties in determining the optimal annealing temperature can be overcome in many cases by performing touchdown PCR.
RT-PCR cycling conditions not optimal	Using the same cycling conditions, repeat the RT-PCR using Band Clear Solution..
Primer concentration not optimal or primers degraded	A primer concentration of 0.6 μM is strongly recommended. However, if the desired results are not obtained using this concentration, repeat the RT-PCR with different primer concentrations from 0.5–1.0 μM in 0.1 μM increments.
Contamination with genomic DNA	Pretreat starting RNA template with DNase I.
Smeared Product	**Comments and suggestions**
Too much starting template	Check the concentration of the starting RNA template. If necessary, make new serial dilutions of template RNA from stock solutions. Repeat the RT-PCR using the new dilutions.
Carry-over contamination	If the negative control (without template RNA) shows a RT-PCR product or a smear, exchange all reagents. Use disposable pipet tips containing hydrophobic filters to minimize crosscontamination. Set up all reaction mixtures in an area separate from that used for RNA preparation or PCR product analysis.
Too many cycles	Reduce the number of cycles in steps of 3 cycles.
Primer concentration not optimal or primers degraded	A primer concentration of 0.6 μM is strongly recommended. However, if the desired results are not obtained using this concentration, repeat the RT-PCR with different primer concentrations from 0.5–1.0 μM in 0.1 μM increments.

■Memo

Date issued : Nov. 30, 2009 Doc. No.: I 5001-5

Manufactured by **BIONOTE, Inc.**
2-9, Seogu-dong, Hwaseong-si, Gyeonggi-do, Korea, 445-170
TEL: 82-31-211-0516 FAX: 82-31-8003-0618,
http://www.bionote.co.kr