Cat. # RR024A

For Research Use

TaKaRa

TaKaRa One Step RNA PCR Kit (AMV)

Product Manual

v202006Da

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I. Description

PCR (Polymerase Chain Reaction) process is a simple and powerful method which allows *in vitro* amplification of DNA fragments through a succession of three incubation steps at different temperatures. In principle, PCR is a method to amplify DNA segments, and not directly amplify RNA. However, synthesis of cDNA from RNA using reverse transcriptase enables to apply PCR process to the RNA analysis. Many reports of various fields have been made by applying this method, such as of structural analysis of RNA, efficient cDNA cloning, analysis of gene expression at the RNA level, etc.
This kit is designed to perform the reverse transcription of RNA to cDNA using AMV (Avian Myeloblastosis Virus) Reverse Transcriptase and subsequent amplification using *AMV-Optimized Taq* (which was developed in LA (Long and Accurate) PCR technology.) all in a single tube. Conventional RT-PCR kits employ a 2 step RT-PCR, which is performed sequential reactions and separately prepared reactions mixtures. These addtional steps are undesirable, as they increase the risk of contamination. One-step RNA-PCR Kit minimizes the risk of contamination, and the supplied *AMV-Optimized Taq* allows LA PCR. Included are all reagents necessary for the reverse transcription and subsequent cDNA amplification, so this kit allows simple and efficient analysis of RNA.

II. Principle

This kit allows reverse transcription from RNA to cDNA using AMV RTase and subsequent amplification in the same tube utilizing *AMV-Optimized Taq* DNA Polymerase.

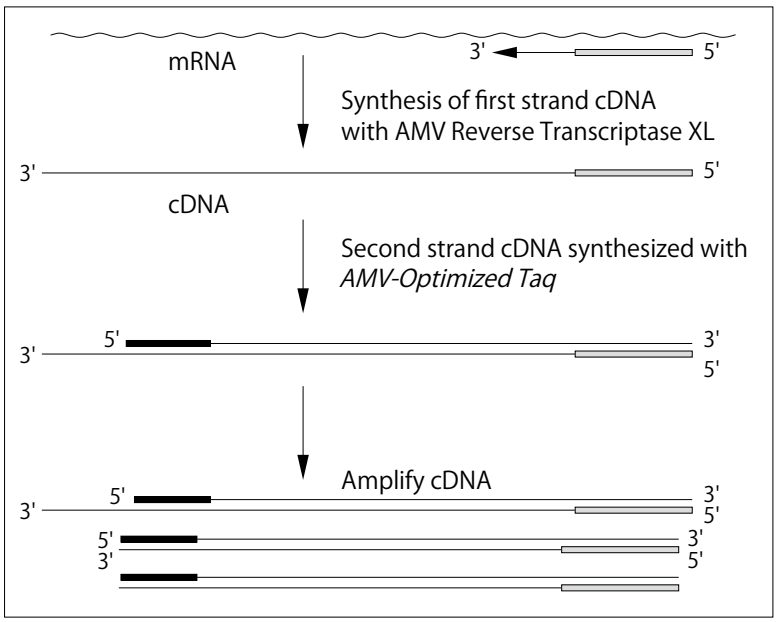


Figure 1. Principle of One Step RT-PCR

III. Features

Template RNA	General
Amplified size	At least 5.6 kb
Reverse Transcriptase	AMV Reverse Transcriptase XL (in the range of 42 - 60℃)
Primer for reverse transcription	Specific downstream primer (antisense primer for PCR) (Not use Oligo dT primer and Randam primer.)
DNA Polymerase	*AMV-Optimized Taq*
RNase Inhibitor	Supplied in the kit
Protocol	Single tube reaction (RTase is heat inactivated prior to PCR.)

IV. Components (50 reactions)

1.	AMV Reverse Transcriptase XL (originated from Avian Myeloblastosis Virus)	(5 units/ μl)	50 μl
2.	RNase Free dH_2O		1 ml x 2
3.	RNase Inhibitor	(40 units/ μl)	50 μl
4.	*AMV-Optimized Taq*	(5 units/ μl)	50 μl
5.	10X One Step RNA PCR Buffer		250 μl
6.	dNTP Mixture	(ea. 10 mM)	250 μl
7.	$MgCl_2$	(25 mM)	500 μl
8.	Control F-1 Primer*1 (upstream primer for Positive Control RNA)	(20 pmol/ μl)	25 μl
9.	Control R-1 Primer*1 (downstream primer for Positive Control RNA)	(20 pmol/ μl)	25 μl
10.	Positive Control RNA*2 (polyA$^+$ RNA transcribed from pSPTet3 plasmid)	(2X 10^5 copies/ μl)	25 μl

*1 Primer Sequence
Control F-1 Primer: 5′-CTGCTCGCTTCGCTACTTGGA-3′
Control R-1 Primer: 5′-CGGCACCTGTCCTACGAGTTG-3′

*2 Supplied control RNA is *in vitro* transcribed RNA using SP6 RNA polymerase from plasmid pSPTet3 inserted with DNA fragment (approximately 1.4 kb) having tetracycline resistant gene, originated from pBR322, in the downstream of SP6 promoter.

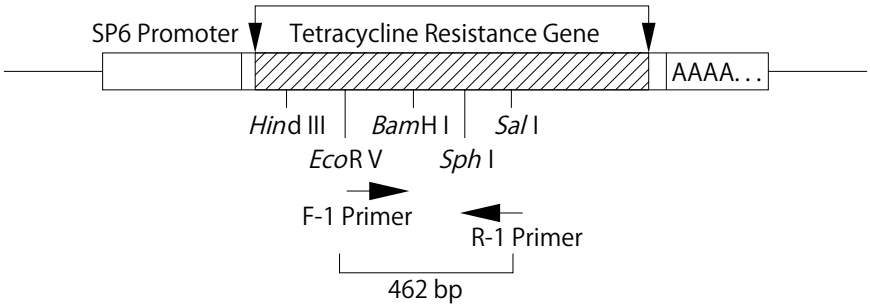


Figure 2. Amplified DNA fragment using Positive Control RNA and primers

V. Materials Required but not Provided

1. Agarose gel
   ex. PrimeGel™ Agarose PCR-Sieve (Cat. #5810A)
   PrimeGel Agarose LE 1-20K GAT (Cat. #5801A)
2. Authorized instruments for PCR
3. Microcentrifuge tubes (made of polypropylene)
4. Agarose gel electrophoresis apparatus
   ex. Mupid-exU, Mupid-2plus (Mupid CO., LTD)
5. Microcentrifuge
6. Micropipetts and pipette tips (autoclaved)

VI. Storage -20℃

VII. Protocol

VII-1. Preparation of RNA sample

This kit is designed to perform the reverse transcription of RNA to cDNA and subsequent amplification. The purity of RNA sample will affect the yield of cDNA synthesis. So it is essential to inhibit the activity of RNase in the cells and also to prevent the contamination of RNase derived from equipments and solutions used. Extra precautions should be taken during the sample preparation; put on clean disposable gloves, dedicate a table to exclusive use for RNA preparation, and avoid unnecessary talks during the operations to prevent the contamination of RNase from operators' sweat or saliva.

[Equipment]

Disposable plastic equipments should be used. In case using glass tools, treat the glass tools with DEPC (diethylpyrocarbonate) prior to use.

(1) Treat glass tools with 0.1% DEPC solution at 37℃ for 12 hours.
(2) Autoclave at 120℃ for 30 min to remove DEPC left on the tools.

It is recommended to prepare all the equipments as the exclusive use for RNA preparation.

[Reagent]

Reagents for RNA preparation, including distilled water, should be prepared with heat sterilized glass tools (180℃, 60 min), or if possible those treated with 0.1% DEPC solution and autoclaved. Reagents and distilled water should be exclusively used for RNA preparation.

[Preparation method]

Simple purification methods can yield enough amount of RNA for reverse transcription and subsequent PCR. However, it is recommended to use highly purified RNA obtained by GTC (Guanidine thiocyanate) method, etc.

VII-2. Note

1. For both reverse transcription and PCR amplification, master mix of reagents (containing RNase Free dH_2O, buffers, dNTP Mixtures, $MgCl_2$ solution, etc.) for all samples can be prepared first, then aliquoted to individual tubes. Using such mixtures will allow accurate reagents dispense: minimize reagents pipetting losses, and avoid repeat dispensing of the each reagent. This helps to minimize variation of the data among the experiments.

2. Enzymes such as RTase, *AMV-Optimized Taq* and RNase Inhibitor shall be mixed gently by pipetting. Avoid generating bubbles. Gently spin down the solution prior to mixing. Pipette enzymes carefully and slowly as the viscosity of the 50% glycerol in the buffer can lead to pipetting errors.

3. Keep enzymes at -20℃ until just before use and return into the freezer promptly after use.

4. Please avoid freeze-thawing cycle for the Positive Control RNA. It is recommended to store the Positive Control RNA in aliquots at -70 ~ -80℃.

5. Use new disposable pipette tips to avoid contamination between samples.

6. Please use Specific downstream primer for reverse transcription. Not use Random Primer and Oligo dT Primer.

VII-3. Standard RT-PCR protocol

1. Prepare the reaction mixture.

Reagents	Volume	Final conc. (or Volume to be added)
10X One Step RNA PCR Buffer	5 μl	1X
25 mM $MgCl_2$	10 μl	5 mM
10 mM dNTP Mixture	5 μl	1 mM
RNase Inhibitor (40 units/μl)	1 μl	0.8 units/μl
AMV RTase XL (5 units/μl)	1 μl	0.1 unit/μl
AMV-Optimized Taq (5 units/μl)	1 μl	0.1 unit/μl
Specific upstream PCR primer (20 μM)*1	1 μl	0.4 μM
Specific downstream PCR primer (20 μM)*2	1 μl	0.4 μM
Positive Control RNA or Experimental Sample*3	1 μl	1 μg of total RNA
RNase Free dH_2O	24 μl	
Total	50 μl	

*1 In case of Positive Control RNA, use the control F-1 Primer.

*2 In case of Positive Control RNA, use the control R-1 Primer.
Specific downstream PCR primer is required for reverse transcription using this kit. (Random primer or oligo dT primer cannot be used for this kit.)

*3 In case the amount of sample RNA is small, the total volume of the sample can be added up to 25 μl.

2. Place all tubes in a Thermal Cycler and set the parameters by following condition.

[Standard condition]			[In the case of Positive Control RNA]		
50℃	30 min	Reverse transcription	50℃	15 min	
94℃	2 min	Inactivation of RTase	94℃	2 min	
94℃	30 sec	PCR 25 - 30 cycles	94℃	30 sec	28 cycles
37 - 65℃	30 sec		60℃	30 sec	
72℃	1-10 min		72℃	1.5 min	

3. After the amplification is completed, apply 5 - 10 μl of the reactant for agarose gel electrophoresis to verify the amplified DNA fragments. The PCR amplified product samples can be stored frozen until subsequent analysis. In the control experiment target cDNA can be verified by the amplified fragment of 462 bp.

VIII. Application

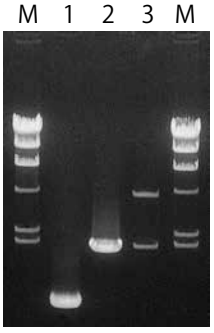
VIII-1. Amplification of cDNA fragments of TFR regions (1.0, 2.0, and 4.4 kb) from the total RNA derived from HL60 cell

1. RT-PCR mixture is prepared as "VII-3. Standard RT-PCR protocol".

2. After preparation of the RT-PCR mixture, place all tubes in Thermal Cycler and set the parameters by the following condition.

50℃	30 min	
94℃	2 min	
94℃	30 sec	30 cycles
60℃	30 sec	
72℃	6 min	

3. After the amplification is completed, apply 4 μl of the reactant for agarose gel electrophoresis to verify the amplified DNA fragments.



1：TFR region 1 kb
2：TFR region 2 kb
3：TFR region 4.4 kb
M：λ-*Hin*d III digest

1.0% agarose gel

VIII-2. PCR condition

● Temperature of annealing
In the case of the Positive Control RNA annealing is carried out at 60℃, but this temperature may change depending on an experimental sample. It is necessary to determine optimal annealing temperature experimentally by varying temperatures in the range of 37 - 65℃.

● Extension time
The extension time depends on the length of the target cDNA. Usually *AMV-Optimized Taq* extends DNA at 1 - 2 minutes per 1 kb at 72℃.

● Number of cycle
The 40 - 50 cycles are recommended in the case that cDNA contents is low.

VIII-3. PCR products

Most of the PCR products amplified using this kit have a 3′ A overhang.
Therefore, it is possible to clone the PCR product directly into a T-Vector.
In addition, it is possible to clone into a blunt end vector by blunting the ends and phosphorylation. This can be done using the Mighty Cloning Reagent Set (Blunt End) (Cat. #6027) for blunt end vector cloning.

IX. References

1) Kawasaki, E. S. and Wang, A. M. *PCR Technology* (Erlich, H. A. ed). *Stockton Press*. (1989) 89-97.
2) Lynas, C., Cook, S. D., Laycock, K. A., Bradfield, J. W. B., and Maitland, N. J. *J Pathology*. (1989) **157**: 285-289.
3) Frohman, M. A., Dush, M. K., and Martin, G. R. *Proc Natl Acad Sci USA.* (1988) **85**: 8998-9002.
4) Mallet, F., Oriol, G., Mary, C., Verrier, B., and Mandrand, B. *BioTechniques.* (1995) **18**(4): 678-687.

X. Related Products

Reverse Transcriptase XL (AMV) for RT-PCR (Cat. #2630A)
Recombinant RNase Inhibitor (Cat. #2313A/B)
Mighty Cloning Reagent Set (Blunt End) (Cat. #6027)
TaKaRa PCR Thermal Cycler Dice™ Gradient (Cat. #TP600)
TaKaRa PCR Thermal Cycler Dice™ *Touch* (Cat. #TP350)*

* Not available in all geographic locations. Check for availability in your area.