6. 反应中 $MgCl_2$ 浓度可依据不同条件进行调整；
7. 引物的设计的好坏直接影响到 RT-PCR 反应的结果，设计引物考虑多种因素，如 GC 含量，引物长度，引物位置等因素，因此我们建议采用专业的引物设计软件来设计，如 Primer Premier 5.0 等。

实验操作 Protocol

反应液按以下条件配制

10 x RT-PCR Buffer	2.5 µl
dNTP Mixture	4 µl
RNase inhibitor	1 µl
Upstream primer(10 µM)	1 µl
Downstream primer(10 µM)	1 µl
AMV reverse transcriptase	0.5 µl
Taq polymerase	0.5 µl
总 RNA <1ug	X µl
RNase free H_2O	14.5-X µl
总体积	25 µl

反应液可等比扩大到 50ul，反应体系各组份也须等比放大。

45℃*	30min	
94℃	5min	
94℃	30s	30-40cycles***
50-65℃**	30s	
72℃	1kb/min	
72℃	5min	

取 5 µl RT-PCR 产物进行琼脂糖凝胶电泳

说明：

* 对于有复杂二级结构的 RNA 模板，反应温度可适当提高

** 根据引物 Tm 值调整，一般为 Tm-5℃

***如果 RNA 量少，可适当增加循环数（45-50 循环）

参考文献

5. Houts, G.E., Miyagi, M., Ellis, C., Beard, D., and Beard, J.W. (1979) *J.Virol.* 29(2):517-522.
6. Guide to Molecular Cloning Techniques. Methods in Enzymology, Volume 152. pp 316-325. Edited by Shelby Berger and Alan R. Kimmel. Academic Press, Inc.
7. Aatsinki JT, Lakkakorpi JT, Pietila EM, Rajaniemi HJ. *Biotechniques.* 1994 ;16(2):282-4, 286-8.
8. F. X. Limbach, B. Jaulhac, Y. Pie´ Mont, J. L. Kuntz, H. Monteil, and J. Siblia. *J Clin Microbiol.* 1999. 2037–2039.

BioRT One Step RT-PCR Kit

BioRT 逆转录扩增(RT-PCR)试剂盒说明书 (一步法)

Cat No.: BSB07M1

TECHNICAL SUPPORT:

For technical support, please dial phone number ：

0086-571-87774567-5278 or 5211, fax to 0086-571-87774303

email to reagent@bioer.com.cn.

Website: www.bioer.com.cn

3. AMV reverse transcriptase, Taq polymerase and RNase inhibitor should be slowly pipetted after centrifuging. The remainder is better to store at -20℃ as soon as possible;
4. Avoid frequently freezing and thawing dNTP;
5. Specific primers should be used and concentration of primers should be optimized and We suggest 0.4μM as a starting point for optimizing; Oligo-dT and Random primers are not suitable for this kit;
6. 2mM $MgCl_2$ is already included in 10*RT-PCR buffer. The concentration of $MgCl_2$ can be adjusted depending on different conditions. 2.5-3mM is suggested when the length of target template is longer than 2kb;
7. The primer design directly effects the performance of RT-PCR. Factors such as GC percentage, length of primers and site of primers sites should be considered. We suggest using software, such as Primer Premier5.0.

Protocol

Prepare the reaction mix by combining the indicated components

10 x RT-PCR Buffer	2.5 μl
dNTP Mixture	4 μl
RNase inhibitor	1 μl
Sense primer(10 μM)	1 μl
Antisense primer(10 μM)	1 μl
AMV reverse transcriptase	0.5 μl
Taq polymerase	0.5 μl
Total RNA <1ug	X μl
RNase free H_2O	14.5-X μl
Total Volume	25 μl

The reaction system can be enlarged to 50μl in accordance with the corresponding proportion.

REACTION	CONDITIONS	
42℃*	30min	
94℃	5min	
94℃	30s	30-40cycles***
50-65℃**	30s	
72℃	1kb/min	
72℃	5min	

Analyze 5μl of the reaction products by agarose gel electrophoresis

Notes

* Reaction temperature can be elevated moderately for RNA template with second structure
** 5℃ lower than Tm value of primers
*** Add to 45-50 cycles when detecting rare RNA templates

References

1. Houts, G.E., Miyagi, M., Ellis, C., Beard, D., and Beard, J.W. (1979) *J.Virol.* 29(2):517-522.
2. Guide to Molecular Cloning Techniques. Methods in Enzymology, Volume 152. pp 316-325. Edited by Shelby Berger and Alan R. Kimmel. Academic Press, Inc.
3. Aatsinki JT, Lakkakorpi JT, Pietila EM, Rajaniemi HJ. *Biotechniques.* 1994 ;16(2):282-4, 286-8.
4. F. X. Limbach, B. Jaulhac, Y. Pie´ Mont, J. L. Kuntz, H. Monteil, and J. Siblia. *J Clin Microbiol.* 1999. 2037–2039.

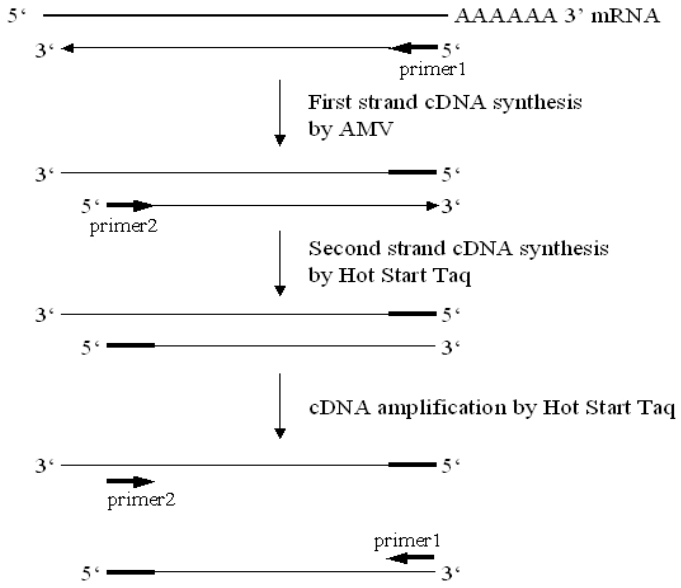
产品说明

RT-PCR 是指利用逆转录酶将 RNA 逆转录(RT)成 cDNA(Complementary DNA)，然后以 cDNA 为模板，通过聚合酶链式反应(PCR)扩增目的片段的技术。RT-PCR 技术可用于检测细胞和组织中基因表达水平，克隆特定基因的 cDNA 序列和检测 RNA 病毒。

BioRT 逆转录扩增(RT-PCR)试剂盒(一步法)采用一步法使 RT 和 PCR 在同一反应体系中进行，反应过程中不需要添加试剂，本试剂盒采用美国先进技术生产的高质量逆转录酶(AMV 酶)和热启动 Taq 聚合酶，并采用特殊的反应体系保证 AMV 逆转录酶和热启动 Taq 酶发挥最大功效。AMV 逆转录酶可逆转录得到高产量的 cDNA，热启动 Taq 聚合酶采用高度纯化的重组 Taq 聚合酶和单克隆抗体相结合，具有高特异性，高灵敏，高延伸速度等特点。

本试剂盒采用一步法进行，即 RT 和 PCR 分别在同一管中进行，反应过程中无需打开管盖，避免了污染，同时提高了检测的灵敏度。

RT-PCR 原理



Kit Components

(100 rxns)

AMV Reverse Transcriptase(5U/μl)	50 μl
Taq polymerase(5U/μl)	50 μl
10X RT-PCR Buffer	500 μl
dNTP Mixture(2.5mM)	500 μl
$MgCl_2$(25mM)	500 μl
RNase inhibitor(10U/μl)	100 μl
RNase free H_2O	1000 μl×2
RNA control(100ng/μl)	20 μl
RNA control S primer(10μM)	20 μl
RNA control A primer(10μM)	20 μl

Store at -20℃

Required materials for RT-PCR

Instruments and consumables	Reagents
Centrifuge	DEPC
Pipettes	ddH_2O
RNase free 1.5ml tubes	Electrophoresis Buffer
Water bath instruments	Loading Buffer
Gel electrophoresis instruments	DNA Marker
PCR tubes	
Tips	

Usage Tips

1. Total RNA or mRNA can be used as RNA template，and we suggest using Biozol(BSC51M1)to isolate high quality RNA；
2. RNase contamination should be avoided:
   1） Wear one-off gloves and respirator because of the Rnases in saliva and skin;
   2） Use special instruments and consumables and handle in specific areas;
   3） Consumables should be dry heat sterilized at 180℃ for 60min, or treat at 37℃ with0.1% DEPC aqueous solution for 12 hours followed by sterilization at 121℃ for 30min.

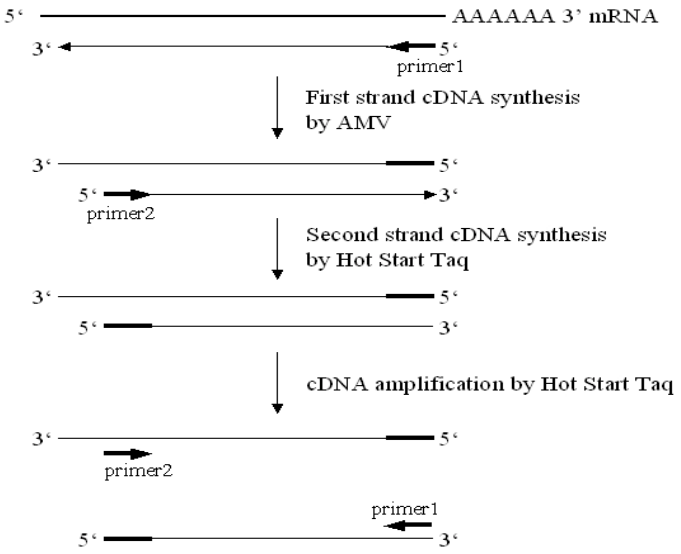
Description

RT-PCR (Reverse Transcription-Polymerase Chain Reaction) is a technique in which an RNA strand is "reverses" transcribed into its DNA complement, followed by amplification of the resulting DNA using a polymerase chain reaction (PCR). RT-PCR can be used to examine gene expression level in cells and tissues, clone the specific gene of cDNA sequences and test RNA viruses.

Unlike two steps RT-PCR methods, in which RT and PCR separated in two tubes, BioRT One Step RT-PCR Kit is designed to combine RT and PCR in the same response system. No additional reagents are needed during the process. Our kits adopt High-quality AMV reverse transcriptase produced in USA and Taq polymerase. We introduce an optimized buffer to ensure the maximize effectiveness of these two enzymes. Cooperation of AMV reverse transcriptase and highly purified recombinant Taq polymerase can obtain 4kb extent of products

BioRT One Step RT-PCR Kit adopts one tube system. Because operator doesn't need to open the lid during the reaction process, this user-friendly improved version avoids cross contamination and has the merits of high specificity and sensitivity.

One Step RT-PCR Principle



试剂盒组成

(100 次使用量)

AMV Reverse Transcriptase(5U/μl)	50 μl
Taq polymerase(5U/μl)	50 μl
10X RT-PCR Buffer	500 μl
dNTP Mixture(2.5mM)	500 μl
$MgCl_2$(25mM)	500 μl
RNase inhibitor(10U/μl)	100 μl
RNase free H_2O	1000 μl×2
RNA control(100ng/μl)	20 μl
RNA control S primer(10μM)	20 μl
RNA control A primer(10μM)	20 μl

保存　　-20℃

RT-PCR 实验必需用品

仪器和耗材	试剂
离心机	DEPC(焦碳酸二乙酯)
微量移液器	ddH_2O
RNase free 1.5ml 离心管	电泳缓冲液
水浴装置或金属浴装置	上样缓冲液
电泳及 UV 装置	DNA Marker
PCR 管	
移液器吸头	

使用提示

1. RNA 模板可以采用总 RNA 或 mRNA，建议使用 Trizol， Biozol 或 RNA 离心柱制备高质量 RNA；
2. 一步法 RT-PCR 实验应避免 RNase 污染，可采用以下措施：
   1） 因人的皮肤表面和唾液都有 RNase，因此实验中应戴一次性手套和口罩；
   2） 一步法 RT-PCR 实验应使用专门的仪器和耗材，建议在专门区域操作 RNA；
   3） 一步法 RT-PCR 实验相关耗材应使用干热灭菌（180℃，60 分钟）或用 0.1% DEPC(焦碳酸二乙酯)水溶液在 37℃处理 12 小时后在 121℃高压灭菌 30 分钟；
3. AMV 逆转录酶，Taq 聚合酶和 RNase 抑制剂在取用之前应离心后再吸取，吸取时动作要慢，使用后应尽快放回-20℃；
4. dNTP 应避免反复冻融以免失效；
5. 本试剂盒必须使用特异性引物，引物的选择可根据具体情况，Oligo-dT 引物和随机引物不适用于本试剂盒；