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BIO BASIC INC.

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One-Step M-MuLV RT-PCR Kit

BS665
QF 24 TV4
Cv2 2025

For Research Use Only

One-Step M-MuLV RT-PCR Kit
Code: BS665 (100 Preps)

Description

Bio Basic's One-Step M-MuLV RT-PCR kit offers a unique system for performing RT-PCR in a single step, in a single tube. Traditionally, RT-PCR is performed in two reaction steps. In the initial reaction, primary-strand cDNA is reverse transcribed from total or poly(A) RNA. Then, in a separate reaction, the cDNA is amplified by PCR using a Taq DNA polymerase. This kit allows cDNA synthesis and PCR to be performed in a single optimized buffer, with both M-MuLV RT and Taq DNA polymerases. No additional reagents are required after the reaction is initiated. This method reduces the possibility of cross-contamination and provides a very convenient technique for gene expression.

Low Specificity

A. Reaction conditions not optimal

- Optimize Mg concentration.
- Optimize primer.
- Optimize annealing temperature and extention time.
- Increase temperature of RT to 40-42°C.

B. Oligo-dT or random primers used for 1st strand synthesis

Use gene specific primers.

Unexpected Bands

A. RNA contamination with genomic DNA

Pre-treat RNA with DNAse I.

PRODUCTS ARE FOR SCIENTIFIC RESEARCH ONLY
NOT INTENDED FOR HUMAN OR ANIMAL USE

Troubleshooting

No Amplification Product

A. No cDNA Synthesis (temperature too low)

For cDNA synthesis step, incubate at 50°C.

B. RNA Contamination

Maintain RNAse-free conditions, add RNAse inhibitor.

C. Not enough starting material of RNA

Increase RNA concentration, use > 1ug of total RNA.

D. RNA has been damaged

Replace RNA.

E. RT inhibitors are present in RNA

Remove inhibitors in RNA preparation by additional 70% ethanol wash.

NOTE: Inhibitors include SDS, EDTA, spermidine, etc.

F. Annealing Temperature too high

Decrease temperature.

G. Extension time is too short

Set extension time for at least 1 minute per kb.

H. Cycle number is too low

Increase number of cycles.

Features

- Rapid and convenient.
- Simple procedure. No transfer of sample between tubes is needed, which minimizes cross-contamination.
- Easy to detect more than one sample.
- 1.2 mM final concentration of magnesium in the reaction mix works well for most targets. If needed, the magnesium concentration can further be optimized (usually between 1.2-2 mM) with the 5 mM MgSO4 solution provided.

NOTE: Care must be taken when working with RNA. It is important to maintain an RNAse-free environment starting with RNA sample preparation and continuing through all purification and analysis. Use RNAse free tubes, tips, and gels. Wear gloves at all times.

Kit Contents

Components	BS665 (100 Preps)
10× One-Step RT-PCR Buffer	1 ml
M-MULV RT	50 µl
Taq DNA polymerase	50 µl
RNase Inhibitor (40 U/µl)	50 µl
RNase free ddH_2O	2 ml
$MgCl_2$ (5 mmol/L)	1 ml
Protocol	1

Storage

Transportation at 4°C. Store at -20°C. Valid for 1 year.

Quality Control

This product is tested functionally using 12.5 pg of total HOX RNA as a template for the amplification of a 674-bp segment of beta-actin mRNA (40 cycles). A minimum of 25 ng of the RT-PCR product was obtained under these conditions.

Procedure

1. Add RNase free ddH_2O, RNA, 10X One Step RT-PCR Buffer, gene specific primers, M-MULV RT, Taq DNA polymerase into a sterile, nuclease-free tube on ice in the order indicated below:

Component	Volume (for 50 µl Reaction)	Final Concentration
10× One-Step RT-PCR Buffer	5 µl	1X
Forward primer (10 µmol/l)	2 µl	0.4 µmol/l
Reverse primer (10 µmol/l)	2 µl	0.4 µmol/l
M-MULV RT	0.5 µl	------
Taq DNA Polymerase	0.5 µl	------
RNAse Inhibitor (40 U/µl)	0.5 µl	------
Total RNA	x µl (<1 µg)	10pg – 1µg
RNase free water	Fill to 50 µl	------

2. Mix gently, centrifuge briefly and place on ice.

3. Run the reactions using a thermocycler.

Recommended Program Settings

42°C	10~30 minutes	cDNA synthesis
94°C	5 minutes	pre-denaturation
94°C	30 seconds	30~40 cycles
40~65°C	30 seconds	
72°C	1 kb/minute	
72°C	10 minutes	

4. Setting the PCR parameters.

   a. The optimal number of cycles depends on transcript abundance and template complexity, and must be determined empirically.

   b. For experimental reactions, use 1 minute of extension time per kb.