BIO BASIC
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Product information

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One-Step AMV RT-PCR Kit

Catalog #: BS6649
Size: 100 preps
Storage: -20°C*

*: Product shipped with ice pack. Check storage conditions.

Product Description:

One-Step AMV RT-PCR 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, first- 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 AMV RT and Taq DNA polymerase. 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.

Features:

- Rapid and convenient.
- Simple procedure. No transfer of sample between tubes is needed, which minimizes the cross-contamination.
- Easy to scale up.
- AMV RT has high activity at 37°C ~55°C.
- 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 $MgCl_2$ solution provided.

Composition:

10× One-Step RT-PCR Buffer	1 ml
AMV RT	50 µl
Taq DNA polymerase	50 µl
RNase inhibitor	50 µl
RNase free ddH_2O	2 ml
$MgCl_2$ (5 mmol/l)	2 ml
Protocol	1

Storage and Transportation:

Transportation with an ice pack. Store components at -20°C.

Procedures:

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

10× One-Step RT-PCR Buffer	5 µl
Forward primer (10 µmol/l)	2 µl
Reverse primer (10 µmol/l)	2 µl
AMV RT	0.5 µl
Taq DNA Polymerase	0.5 µl
RNase Inhibitor	0.5 µl
Total RNA	x µl(< 1 µg)
RNase free ddH_2O	up to 50 µl

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

3. Run the reactions. Commence thermal cycling using the following program:

42°C	10~30 minutes
94°C	5 minutes
94°C	30 seconds
40-65°C	30 seconds
72°C	1 kb/minute
72°C	10 minutes

} 30~40 cycles

4. Set the PCR parameters

A) 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.

Troubleshooting Guide:

No Amplification Product

1. No cDNA synthesis (temperature too high): For cDNA synthesis step, incubate at 50°C.
2. RNA Contamination: Double RNase inhibitor.
3. Not enough starting material of RNA: Increase RNA concentration, use 100 mg to 1 µg of total RNA.
4. RNA has been damaged: Replace RNA.
5. RT inhibitors are present in RNA: Remove inhibitors in RNA preparation by additional 70% ethanol wash.

   NOTE: Inhibitors include SDS, EDTA, spermidine, etc (4,5).
6. Annealing temperature is too high: Decrease temperature.
7. Extension time is too short: Set extension time for at least 1 minute per kb.
8. Cycle number is too low: Increase number of cycles.

Low Specificity

1. Reaction conditions not optimal: Optimize Mg concentration. Optimize primer. Optimize annealing temperature and extention time.
2. Oligo-dT or random primers used for 1st strand synthesis: Use gene specific primers.

Unexpected Bands

1. RNA contamination with genomic DNA: Pre-treat RNA with DNAse I.