FOR RESEARCH USE ONLY!

HiFidelity™ One Step RT Kit

10/20

(Catalog # M1503-100; 100 Rxns; Store at -20°C)

I. Introduction:

Biovision's One-Step RT-PCR Kit is used for the highly sensitive and specific reverse transcription and high fidelity PCR amplification of an RNA template in a single reaction tube. It contains an enzyme mix of highly sensitive Reverse Transcriptase and high fidelity DNA Polymerase with RNaseOFF Ribonuclease Inhibitor, gel loading dye, and all other necessary reagents in a single one-Step 2X RT-PCR Buffer. It provides flexibility in choosing the desired primers for use with a proprietary RT-PCR buffer containing stabilizers and enhancers that optimize the two reactions in a "single step". This kit offers a simple, efficient reaction set-up and is a reliable alternative to conventional "two-step" sequential RT-PCR.

II. Key Features:

- Easy and convenient
- **High sensitivity and high fidelity**
- Reverse transcription and PCR amplification in a **single step**
- Many downstream applications such as gene-expression analysis, transcription analysis, detection assays, etc.

III. Sample Type:

- RNA

IV. Kit Contents:

Components	M1503-100 (100 Rxns)	Part Number
RT-PCR Enzyme Mix	400 µl	M1503-100-1
2X One-Step RT-PCR Buffer	2 x 1.25 ml	M1503-100-2

V. User Supplied Reagents and Equipment:

- Pipettes, Pipette tips
- PCR tubes
- Nuclease free water
- Primers (forward and reverse)
- Total RNA or poly(A) + mRNA
- Agarose
- Ethidium Bromide
- Thermal Cycler

VI. Shipping and Storage Conditions:

The kit is shipped in dry ice. All the components of the kit should be stored at -20°C.

VII. Protocol:

Reactions should be assembled in an RNase-free environment. The use of "clean" pipettors designated for PCR and aerosol-resistant barrier tips are recommended.

1. Thoroughly thaw and mix the individual components before use, and **assemble the reaction** on ice.

Component	Volume
2X One-Step RT-PCR Buffer	25 µl
RT-PCR Enzyme Mix	4 µl
Forward Primer (10 µM)	2.5 µl
Reverse Primer (10 µM)	2.5 µl
Total RNA or poly(A) + mRNA	Variable (1 ng - 2 µg/Rxn)
Nuclease-free H2O	up to 50 µl

2. Gently mix the reaction components and briefly centrifuge.
3. **Thermocycling conditions** for standard PCR are given below:

Step	Temperature	Duration	Cycle(s)
cDNA synthesis	60°C	15 min	1
Initial Denaturation	98°C	30 sec	1
Denaturation	98°C	5-10 sec	25-35
Annealing	50-72°C	10-30 sec	
Extension	72°C	20-30 sec/kb*	
Final Extension	72°C	2 min	1
Holding	4°C	-	1

* 20-30 sec/kb, increase as needed

4. After PCR, maintain the **reaction at 4°C** or store at -20°C until use.
5. **Analyze** the amplification products by agarose gel electrophoresis
6. Visualize by ethidium bromide or SafeImage™ Basic DNA Stain (Cat No. M1193) staining.

VIII. Related Products:

BioVision Product Name	Cat. No.	Sizes
Novo™ cDNA Kit	M1165	25, 100 Rxns
Novo™ Transcriptome cDNA Kit	M1167	25, 100 Rxns
Evo™ cDNA Supermix	M1168	25, 100 Rxns
Novo™ cDNA Supermix	M1169	25, 100 Rxns
Evo™ RT Mastermix	M1170	25, 100, 200 Rxns
Evo™ RT Mastermix (with gDNA Removal)	M1171	100 Rxns
Evo™ RT Mastermix (with cell lysis)	M1172	100 Rxns
Evo™ Reverse Transcriptase	M1173	25, 100 Rxns
Novo™ Reverse Transcriptase	M1174	25, 100 Rxns

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