Easy™ One Step RT Kit-Dye

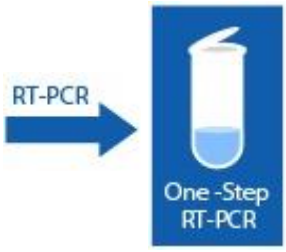
(Cat# M1163-100; One Step RT PCR Kit; Store at -20°C)

I. Introduction:

Easy™ One Step RT Kit contains all the necessary reagents for both reverse transcription and PCR amplification to occur in a single reaction tube. Specifically, this One Step RT PCR kit contains Evo™ Reverse Transcriptase and Distant™ DNA Polymerase in a convenient format for highly sensitive and specific RT PCR using any RNA template. Our proprietary RT PCR buffer contains stabilizers and enhancers that optimize the two reactions in a "single step". Together with a specially formulated RT PCR buffer, this One-Step RT PCR kit offers the end-users an efficient, easy to use and reliable alternative to the conventional "two-step" sequential RT PCR. The dye buffer contains Cresol Red as an electrophoresis dye, with migration equivalent to 125 bp DNA fragment on an agarose gel.

BioVision's Easy™ One Step RT PCR Kit offers the end-user an efficient and easy alternative to the conventional "two step" RT PCR approach with extreme sensitivity, specificity and high product yield.

RNA
Gene-specific Primer
Evo™ RTase
Distant™ DNA Polymerase
2X One Step RT PCR Buffer



II. Application:

- Gene-expression analysis
- Transcription analysis
- Gene cloning
- Multiplex RT PCR
- High throughput applications
- Virus detection and quantification

III. Package Contents (Easy™ One Step RT PCR Kit):

Components	M1163-100 (100 X 50 µl rxns)	Part Number
Distant™ DNA Polymerase (5 U/µl)	200 µl	M1163-XX-1
Evo™ RTase (200 U/µl)	100 µl	M1163-XX-2
2X One Step RT PCR Buffer with Dye	3 X 1 ml	M1163-XX-3

IV. User Supplied Reagents and Equipment:

- PCR Tubes
- Pipettes
- Water, Nuclease-free
- Primers (forward and reverse)
- Total RNA or poly(A) + mRNA

V. Shipment and Storage:

Store all components at -20°C in a non-frost-free freezer. All components are stable for 1 year from the date of shipping when stored and handled properly. Avoid repeated freeze-thaw cycles to retain maximum performance. Briefly centrifuge small vials prior to opening.

VI. Protocol:

RT-PCR should be assembled in a nuclease-free environment. RNA sample preparation, reaction mixture assembly, PCR, and subsequent reaction analysis should be performed in separate areas. The use of "clean", automatic pipettors designated for PCR and aerosol-resistant barrier tips are recommended.

1. Prepare the following reaction mixture in a PCR tube on ice.

Components	Volume	Final Concentration
Total RNA or poly(A) + mRNA	Variable	1 ng - 2 µg/rxn 1 pg - 2 ng/rxn
2X One Step RT PCR Buffer with dye	25 µl	1X
Easy™ RTase (200 U/µl)	1 µl	200 U/rxn
Distant™ DNA Polymerase (5 U/µl)	2 µl	10 U/rxn
Forward Primer (10 µM)	2.5 µl	500 nM
Reverse Primer (10 µM)	2.5 µl	500 nM
Water, Nuclease-free H_2O	Up to 50 µl	-

Note: Gene-specific primers should be used

2. Gently mix and ensure all the components are at the bottom of the amplification tube. Centrifuge briefly if needed.
3. Program the thermal cycler so that cDNA synthesis is followed immediately by PCR amplification automatically. The following cycling conditions were established using a DNA Thermal Cycler 2400 (Perkin Elmer) and may have to be altered for other thermal cyclers.

Steps	Temperature	Duration	Cycle (s)
cDNA Synthesis	42°C	30 min	1
Initial Denaturation	94°C	3 min	1
Denaturation	94°C	30 sec	30 - 35
Annealing	55°C	30 sec	

Extension	72°C	1 kb/min	
Final Extension	72°C	5 min	1
Holding	4°C	-	1

Note: 1) The thermal cycling program listed above is optimized for primers with an annealing temperature at 55°C.
2) An optional touchdown thermal cycling program can also be used to replace the steps after the initial cDNA synthesis in the table above.

4. Analyze the amplification products by agarose gel electrophoresis and visualize the nucleic acids via ethidium bromide. If 2X One Step RT PCR with dye is used, load the samples directly without adding additional loading dye. Use appropriate molecular weight standards.

VII. General Notes:

- Multiple freezing and thawing of RNA should be avoided. Thaw and keep RNA on ice.
- It is recommended that the One Step RT PCR is setup under conditions where RNase contamination has been eliminated. Pipette tips and tubes should be treated with 0.1% DEPC.
- Wearing gloves when performing procedure is highly recommended.

IX. Related Products:

BV Product Name	BV Cat. No.
Two Step RT PCR Kits	M1160-M1161
One Step RT PCR Kits	M1162-M1163
First-Strand cDNA Synthesis Kits	M1164-M1167
First-Strand cDNA Synthesis Supermixes	M1167-M1169
All-In-One RT Mastermixes	M1170-M1172
Reverse Transcriptases	M1173-M1174
One Step Jade™ QRT PCR Kits	M1175-M1182
One Step Taqman QRT PCR Kits	M1183-M1190

FOR RESEARCH USE ONLY! Not to be used on humans.