

Ready One-Step RT-PCR Kit

<u>Code</u>	<u>Description</u>	<u>Size</u>
1B1326-50RXN	Ready One-Step RT-PCR Kit <i>Includes:</i> 1 tube of Ready One-Step Reverse Transcriptase 1 tube of Ready One-Step RT-PCR Mix, 2X	KIT
1B1326-100RXN	Ready One-Step RT-PCR Kit <i>Includes:</i> 1 tube of Ready One-Step Reverse Transcriptase 2 tubes of Ready One-Step RT-PCR Mix, 2X	KIT

General Information:

Ready One-Step RT-PCR Kit offers a single-step procedure for performing RT-PCR reactions. Subsequent loading and visualization of RT-PCR products are streamlined since the loading buffer and visualization dye are included. The user supplies the primers and DNase-treated template RNA.

Ready One-Step RT-PCR Kit includes 1 tube each of Ready One-Step Reverse Transcriptase and Ready One-Step RT-PCR Mix, 2X. Ready One-Step Reverse Transcriptase consists of M-MLV and a proprietary buffer that delivers robust cDNA synthesis from rare and abundant transcripts. Ready One-Step RT-PCR Mix, 2X consists of a mixture of reaction buffer, AMRESCO's Extender™ Taq polymerase blend, dNTPs, an electrophoresis tracking dye, and a non-mutagenic EZ-Vision® visualization dye. Once amplification is complete, the PCR reaction can be directly loaded and separated on an agarose gel using the magenta-colored tracking dye (migrating at approximately 10bp on a 1% gel) to monitor the migration. After electrophoresis the PCR products are immediately visualized with standard UV illumination without additional post-run staining and destaining steps.

Storage/Stability:

Store at -20°C. Ready One-Step RT PCR Mix, 2X is stable through 15 freeze-thaw cycles.



Product Information

Procedure

1. Thaw primers, DNase-treated template RNA, and Ready One-Step RT-PCR Mix, 2X and place on ice.
2. Assemble 24 µL of nucleic acid mix on ice according to the following table:

Components	Volume (50 µL Reaction)	Final Concentration
RNA sample	1 – 23	> 1 ng
Forward Primer, 10 µM	0.5	0.1 µM
Reverse Primer, 10 µM	0.5	0.1 µM
Nuclease-free Water	As needed	–

3. Heat denature the nucleic acid mix at 65 °C for 5 – 10 minutes and immediately place on ice
4. Assemble the One-Step RT-PCR reactions according to the following table:

Components	Volume (50 µL Reaction)	Final Concentration
Nucleic Acid Mix	24	–
Ready One-Step RT-PCR Mix, 2X	25	1X
Ready One-Step Reverse Transcriptase	1	–

**Note: A control for DNA contamination can be prepared by assembling a reaction that does not contain the Ready One-Step Reverse Transcriptase*

5. Reverse transcribe and amplify the DNA according to the cycle below:

Steps	Time (minutes)	Temperature (°C)
A	5:00	25
B	60:00	42
C	15:00	70
D	2:00	95
E	0:30	95
F	0:30	55 – 65
G	1:00*	68 – 72
Repeat Steps E – G 29 times		
H	7:00	68
I	HOLD	4

**Time should be 1 minute for every 1 KB of expected PCR product size.*

6. Load and separate PCR products on an agarose gel at 5 – 8 V/cm. PCR products can be visualized with a standard ultra-violet light source. Fluorescent dye emits in the blue spectra for documentation.

Frequently Asked Questions

Questions	Answers
Why do I not see any product on my gel?	<ol style="list-style-type: none"> 1. Residual DNase contamination from DNase-treated template RNA 2. RNase contamination in you reaction 3. The yield of the product is too low to be observed by the EZ-Vision® dye. Post-Staining with Ethidium Bromide may be required.
Why does my sample float out of the well?	Reactions were intended to be loaded in to agarose gels in TAE buffer. If the running buffer has a higher density than TAE, the sample may not descend into the well.
Why do I see multiple or unexpected product sizes?	<ol style="list-style-type: none"> 1. Contamination of another target RNA or DNA 2. Incomplete digestion of genomic DNA 3. Multiple RNA target sequences exist 4. Non-specific primer annealing 5. Primer dimers

Related Products

Code	Product
0649-50KU 0649-100KU	DNase I
E633-2KU E633-10KU	Ribonuclease Inhibitor (RNase Inhibitor)
E891-100ML E891-500ML E891-50ML-PUMP E891-100ML-PUMP E891-25PK	NucleasEliminator™ NucleasEliminator Wipes™
N580-30ML N580-100ML N580-200ML N643-KIT	Ribozol™ RNA Extraction Reagent Ribozol™ Plus RNA Purification Kit
N788-KIT	Phenol-Free Total RNA Purification Kit



Product Information

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