

PCRopsis™ Reagent RVD-E

For Research Use Only

REF #: 7833001, 7833025, 7833100 & 78331000

Store at room temperature

INTENDED USE

PCRopsis™ Reagent RVD-E is intended for nucleic acid extraction-free processing of samples on swabs, without the need for transport mediums.

01 INTRODUCTION

PCRopsis™ Reagent RVD-E is engineered to simultaneously elute material from swabs, bind a variety of reverse transcriptase quantitative polymerase chain reaction (RT-qPCR) / PCR inhibitors found in clinical samples, lyse specimens and stabilize nucleic acids in a manner that's compatible with RT-qPCR / PCR. The product consists of a proprietary mixture of peptides, salts, stabilizers, buffers and sodium azide to achieve this task. Reagent RVD-E allows for nucleic acid extraction-free sample processing without performing centrifugations or other sample manipulations, which may introduce errors, contaminants, and/or skew the representation of RNA fragments.

02 PRODUCT SIZE

Catalog Number	Volume
7833001	1 mL
7833025	25 mL
7833100	100 mL
78331000	1000 mL

03 STORAGE & STABILITY

PCRopsis™ Reagent RVD-E is shipped and stored at room temperature. The recommended storage temperature is: 4°C ~ 25°C

04 OVERVIEW OF PROTOCOL



- 1) Add ~100 μ L Reagent RVD-E to tube with test swab
- 2) Vortex for ~30 seconds, 3 times, to elute specimen off swab



Reagent RVD-E



Add ~50 μ L of eluted sample to a thin-walled tube or plate



Heat eluted sample to 95°C as indicated below

Mammalian: 5 minutes
Viruses: 10 minutes
Bacteria: 15 minutes

NOTE: heating for a longer period of time does not negatively affect results

NOTE: samples can be heated in a thermal cycler or heating block



Use processed sample into your desired RT-qPCR / PCR mixture

05 WRITTEN PROTOCOL

1. Thoroughly mix Reagent RVD-E to ensure homogeneity, but avoid creating bubbles unnecessarily
 1. Reagent RVD-E has a hazy, white color when homogenized and normal settlement occurs if not regularly mixed
2. Elute material from swab:
 1. Add ~100 µL of Reagent RVD-E to transport tube with swab
 2. Make sure the swab is at least partially submerged into Reagent RVD-E
 3. Vortex for 30 seconds, 3 times, to elute sample
3. Specimen lysis & nucleic acid stabilization:
 1. Transfer ~50 µL of eluted sample into a thin-walled PCR tube / plate and then cap tube or apply plate sealer to plate to prevent evaporation
 2. Heat at 95°C
 1. Mammalian: 5 minutes
 2. Viruses: 10 minutes
 3. Bacteria: 15 minutes
 4. NOTE: heating for a longer period of time does not negatively affect results
 3. Let cool at room temperature for ~10 seconds before continuing

05 WRITTEN PROTOCOL

4. Pipette up & down to ensure complete mixing
5. Use lysed / stabilized sample in your desired RT-qPCR / qPCR procedure
 1. Lysed / stabilized sample should represent 15%~30% of your final RT-qPCR mixture (i.e., 3~6 μL sample into a total volume of 20 μL) depending on the polymerase used
 2. You might observe increasing PCR inhibition when your PCR mixture consist of >35% processed sample

05 WRITTEN PROTOCOL

Suggested thermocycler parameters for RT-PCR / PCR:

1. Reverse transcription:
 - a. 45°C for 15 minutes (extend to 30 minutes if suboptimal results observed)
 - b. 95°C for 2 minutes

2. PCR amplification (~40 cycles):
 - a. 95°C for 5 seconds
 - b. 55°C for 15 seconds
 - c. 72°C for 15 seconds

3. Hold: 4°C

NOTE:

- When amplifying RNA, a 3-step PCR amplification set-up is recommended over a 2-step PCR amplification set-up
- The suggested cycles, temperature, and heating times mentioned above may be optimized by the user as needed

06 STEP-BY-STEP PROTOCOL WITH FIGURES

Step 1



Gently invert
Reagent RVD-E to ensure
homogeneity

Step 2



Add ~100 μ L Reagent RVD-E
to transport tube containing
test swab

Step 3



Vortex tube with swab
and Reagent RVD-E to
elute specimen off swab

06 STEP-BY-STEP PROTOCOL WITH FIGURES

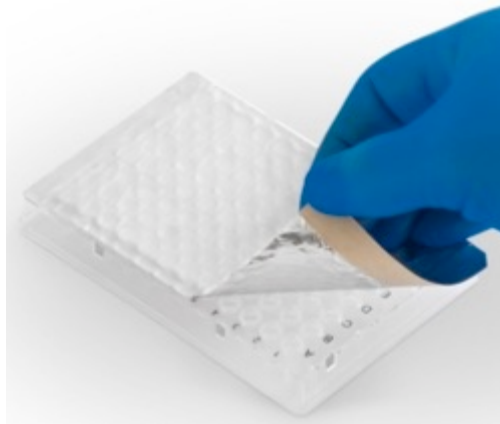
Step 4



Transfer ~50 μ L of eluted sample to thin-walled plate or tube

PCRo^{psis}TM Reagent RVD-E

Step 5



Seal 96-well plate with a plate sealer to prevent evaporation

Step 6

Pre-heated thermal cycler or heating block before applying plate or tube



Recommended heating times:

- Mammalian: 5 minutes
- Viruses: 10 minutes
- Bacteria: 15 minutes

NOTE: heating for a longer period of time does not negatively affect results

Heat eluted mixture at 95°C for recommended time

06 STEP-BY-STEP PROTOCOL WITH FIGURES

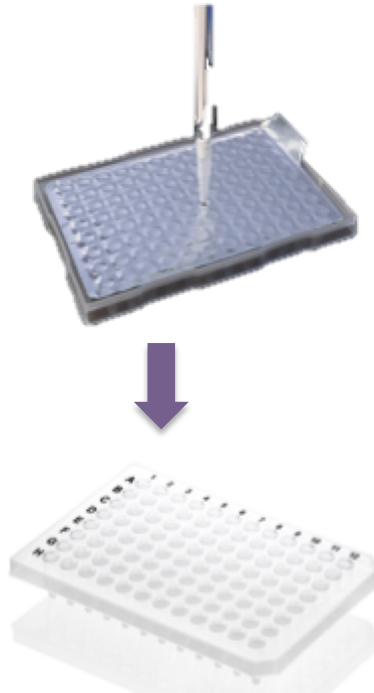
Step 7



Add 15 μ L RT-qPCR mix
from your desired vendor
to a new plate

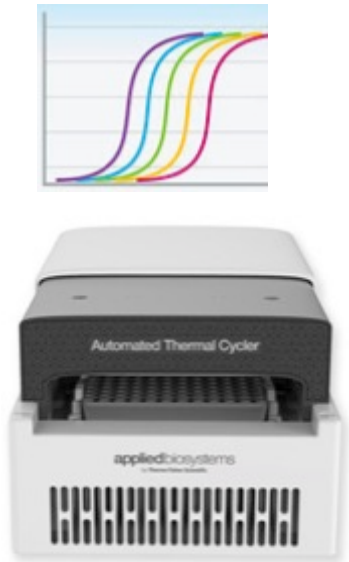
PCRopsis™ Reagent RVD-E

Step 8



Mix 5 μ L of heated, eluted
sample with your
RT-qPCR mix

Step 9



Detect amplification of
target genes using your
desired qPCR equipment

07 TROUBLESHOOTING & SUGGESTIONS

1. Reagent RVD-E is optimized for the amplification of gene targets from specimens on swabs and may not be applicable for other applications.
2. For best results, use recently collected samples that have been properly stored since collection.
3. A complete validation study is warranted when using small synthetic swabs and lower volumes of Reagent RVD-E to improve assay sensitivity.
4. Ct cut-offs for assays should be increased, often times to 40 cycles.
5. Ensure that the processed sample consist of <30% of the total PCR mixture, since high concentrations of processed sample may inhibit PCR for some applications.
6. Take care in maintaining the sterility of your Reagent RVD-E stock after use.
7. Heat Reagent RVD-E / sample mixture for a few minutes longer if you observe suboptimal lysis.
8. It's recommended to use the heated Reagent RVD-E + sample mixture for downstream applications within a day.

08 CONTACT

Contact our research team if assistance with Reagent RVD-E is necessary (info@entopsis.com). We will try our best to assist with non-intended applications of this product or direct you to alternative products. Any business related questions should be directed to: Sales@PCRopsis.com.



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NOT FOR RESALE

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PCRopsis™ Reagent RVD-E

