PCR*opsis*™ Reagent RVD-E

For In Vitro Diagnostic Use

REF #: 7833025, 7833100 & 7833500

REF #: 7833001 (for validation purposes only)

Store at room temperature

INTENDED USE

PCR*opsis*™ Reagent RVD-E is intended for extraction-free amplification of RNA and DNA from specimens on swabs, without the need for transport mediums.

01 INTRODUCTION

PCR*opsis*™ 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 extraction-free amplification of nucleic acids without performing extractions, 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
7833500	500 mL

03 STORAGE & STABILITY

PCR*opsis*™ 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 µL 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 µL 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
   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:

- For most applications, 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 µL Reagent RVD-E to transport tube containing test swab

Step 3

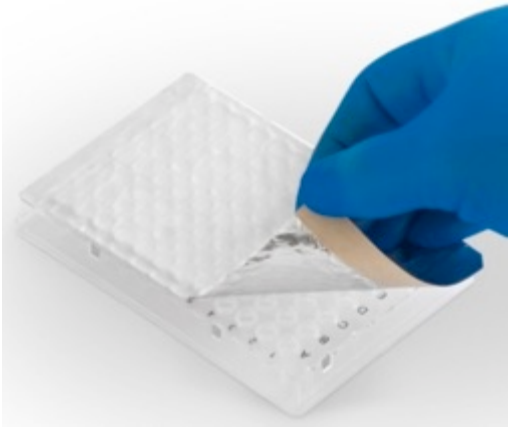
Vortex tube with swab and Reagent RVD-E to elute specimen off swab

06 STEP-BY-STEP PROTOCOL WITH FIGURES

Step 4

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

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

<u>Recommended heating times</u>:

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

<u>NOTE</u>: heating for a longer period of time does not negatively affect results

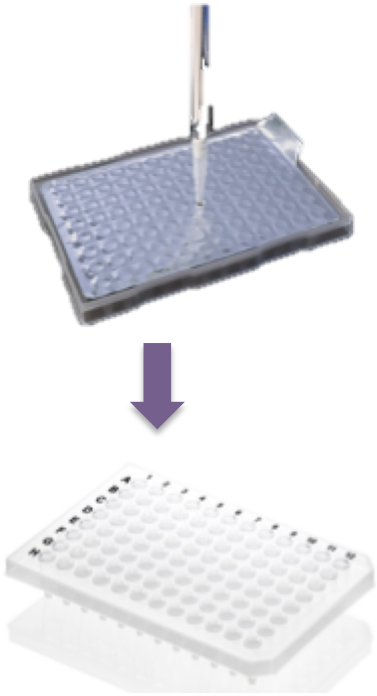
Heat eluted mixture at 95˚C for recommended time

06 STEP-BY-STEP PROTOCOL WITH FIGURES

Step 7

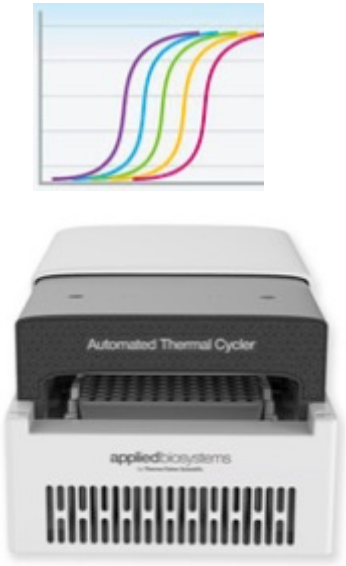
Add 15 µL RT-qPCR mix from your desired vendor to a new plate

Step 8



Mix 5 µL 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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FDA REGISTERED FACILITY

ISO 13485

ISO 9001:2015

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