PCR*opsis*™ Reagent RVD with RVD Enhancer

For In Vitro Diagnostic Use

REF #: 78336025, 78336100, 783361000

REF #: 78336001 (for validation purposes only)

Store at room temperature

INTENDED USE

PCR*opsis*™ Reagent RVD with RVD Enhancer is intended for extraction-free amplification of RNA or DNA from properly collected and transported saliva or urine specimens or swab specimens in compatible transport mediums.

01 INTRODUCTION

PCR*opsis*™ Reagent RVD with RVD Enhancer is engineered to simultaneously bind a variety of reverse transcriptase quantitative polymerase chain reaction (RT-qPCR) and PCR inhibitors found in specimens, lyse cells 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, sodium azide, and RVD Enhancer to achieve these tasks. Reagent RVD with RVD Enhancer allows for extraction-free amplification of RNA / DNA from specimens without performing nucleic acid isolation, centrifugations or other sample manipulations, which may introduce errors, contaminants and/or skew the representation of RNA fragments.

02 PRODUCT SIZE

Catalog Number	Volume
78336001	1 mL
78336025	25 mL
78336100	100 mL
783361000	1000 mL

03 STORAGE & STABILITY

PCR*opsis*™ Reagent RVD with RVD Enhancer is shipped and stored at room temperature. The recommended storage temperature is: 4˚C ~ 25˚C

04 TRANSPORT MEDIUM COMPATIBILITY

RECOMMENDED:

- BD™ Universal Viral Transport System (UVT) - preferred
- Quest V-C-M Medium
- MedSchenker™ Smart Transport Medium
- SORFA Viral Transport Medium
- Mediums with formulations similar to BD™ are expected to be compatible
- Copan ESwab™ Amies Medium
- Phosphate buffered saline (PBS)
- CDC VTM: Hank's Balanced Salt Solution (HBSS) + 2% Fetal Bovine Serum (FBS)
- WHO VTM: Water with veal infusion broth + BSA + antibiotics

NOT RECOMMENDED:

- Mediums containing guanidinium thiocyanate

NOTE: the user must confirm the compatibility of Reagent RVD with RVD Enhancer with desired samples

05 OVERVIEW OF PROTOCOL

Reagent RVD
with RVD Enhancer

Mix 20 µL reagent + 20 µL Sample / well

Heat reagent + sample mixture to 95˚C for 10 minutes

Use processed sample into your desired RT-qPCR / PCR mixture (sample = <30% of total volume)

Test sample

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

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

06 WRITTEN PROTOCOL

1. Thoroughly mix Reagent RVD with RVD Enhancer to ensure homogeneity, but avoid creating bubbles unnecessarily
    1. Reagent RVD with RVD Enhancer has a hazy, white color when homogenized and normal settlement occurs if not regularly mixed
2. Mix 1 volume of sample (20 µL) with 1 volume of Reagent RVD with RVD Enhancer (20 µL) in a thin walled tube (0.2 ~ 0.6 mL) or 96-well PCR plate
3. Pipette up & down to ensure complete mixing and then cap tube or apply plate sealer to plate to prevent evaporation
4. Heat diluted sample at 95˚C and let cool at room temperature for ~10 seconds before continuing
    1. Recommended heating times at 95˚C:
        1. Mammalian: 5 minutes
        2. Viruses: 10 minutes
        3. Bacteria: 15 minutes

        NOTE: heating for a longer period of time does not negatively affect results
    2. Make sure the heating device has reached the desired temperature before applying sample
    3. Sample heating can be performed using a controlled heating block or thermal cycler

06 WRITTEN PROTOCOL

5. Mix heated sample and use lysed / stabilized sample in your desired RT-qPCR / PCR 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

06 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

07 STEP-BY-STEP PROTOCOL WITH FIGURES

Step 1

Gently invert Reagent RVD with RVD Enhancer to ensure homogeneity

Step 2

Add Reagent RVD with RVD Enhancer to reservoir

Step 3

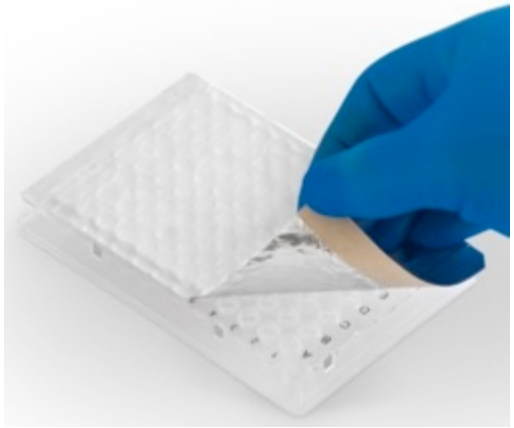
Add 20 µL of reagent to wells in a 96-well PCR plate

07 STEP-BY-STEP PROTOCOL WITH FIGURES

Step 4

Mix 20 µL of sample to wells containing 20 µL of reagent

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

<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

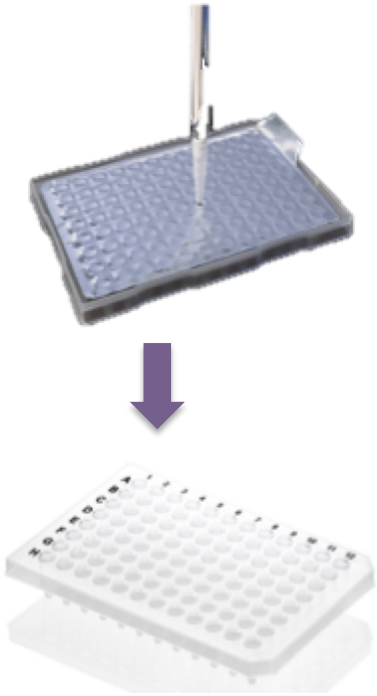
Heat reagent + sample mixture at 95˚C for recommended time

07 STEP-BY-STEP PROTOCOL WITH FIGURES

Step 7

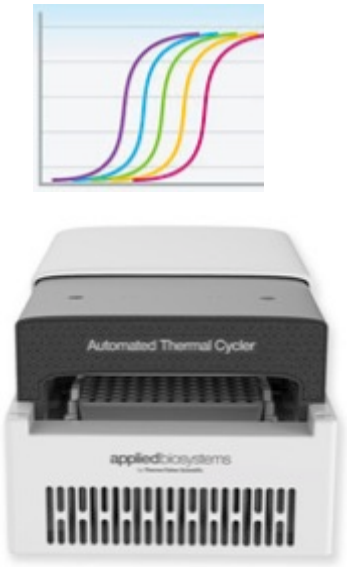
Add 15 µL RT-qPCR mix from your desired vendor to a new plate

Step 8



Mix 5 µL of heated sample + reagent mixture with your RT-qPCR mix

Step 9



Detect amplification of target genes using your desired qPCR equipment

08 TROUBLESHOOTING & SUGGESTIONS

1. Reagent RVD with RVD Enhancer is optimized for the amplification of gene targets from specimens in compatible transport mediums and may not be applicable for other applications or mediums.
2. For best results, use recently collected samples in compatible transport mediums that have been stored at ~4˚C since collection.
3. Ensure that the processed sample consist of <30% of the total PCR mixture, since high concentrations of the processed sample may inhibit PCR for some applications.
4. Take care in maintaining the sterility of your Reagent RVD with RVD Enhancer stock after use.
5. Heat Reagent RVD with RVD Enhancer / sample mixture for a few minutes longer if you observe suboptimal lysis.
6. It's recommended to use the heated Reagent RVD with Enhancer + sample mixture for downstream applications within a day, although samples may be stable for months at 4˚C or -20˚C.

09 CONTACT

Contact our research team if assistance with Reagent RVD with RVD Enhancer 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**.

7600 NW 69th Ave, Medley, FL 33166
www.PCRopsis.com

info@entopsis.com
sales@PCRopsis.com

Toll-free 1-888-407-5070

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