



PCRopsis™ Reagent RVD-RT + PCRopsis™ Activator

For In Vitro Diagnostic Use

REF #: 78378025, 78378100, 7831000

REF #: 78378001 (for validation purposes only)

Store at room temperature

INTENDED USE

PCRopsis™ Reagent RVD-RT is intended for extraction-free amplification of RNA or DNA from properly collected and transported viral specimens in compatible transport mediums at room temperature.

01 INTRODUCTION

PCRopsis™ Reagent RVD-RT, in combination with PCRopsis™ Activator, is engineered to simultaneously bind a variety of reverse transcriptase quantitative polymerase chain reaction (RT-qPCR) and PCR inhibitors found in specimens, 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, sodium azide, and RVD Enhancer to achieve these tasks. Reagent RVD-RT 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
78378001	1 mL
78378025	25 mL
78378100	100 mL
783781000	1000 mL

03 STORAGE & STABILITY

PCRopsis™ Reagent RVD-RT is shipped and stored at room temperature. The recommended storage temperature is: 4°C ~ 25°C. Bring product to room temperature if ice crystals are observed. PCRopsis™ Activator should be stored at 10–25°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 or alcohols
- ◆ Inactivating mediums

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

05 OVERVIEW OF PROTOCOL



+ **PCRopsis™
Activator**

Reagent RVD-RT

Mix 20 μ L
reagent + 20 μ L
Sample / well

Incubate sample
mixture at $\sim 25^{\circ}\text{C}$ for
10 minutes

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



**Test sample
(transport medium, saliva,
centrifuged urine)**

06 WRITTEN PROTOCOL

1. Thoroughly mix PCR*opsis*[™] Reagent RVD-RT to ensure homogeneity, but avoid creating bubbles unnecessarily
 1. Reagent RVD-RT has a hazy, white color when homogenized and normal settlement occurs if not regularly mixed
2. Thoroughly mix 50 µL PCR*opsis*[™] Activator with 950 µL PCR*opsis*[™] Reagent RVD-RT. Called **activated Reagent RVD-RT** from here onwards
 1. This mixture is stable for 24 hours at room temperature in the dark
3. Mix 1 volume of activated Reagent RVD-RT (20 µL) with 1 volume of sample (20 µL) in a sterile tube (0.1 ~ 0.5 mL) or 96-well PCR plate
4. Thoroughly pipette up & down to ensure complete mixing and then cap tube or apply plate sealer to plate to prevent evaporation
5. Incubate sample mixture at room temperature (~25°C) for 10 minutes
6. Immediately mix processed sample and use in your desired RT-qPCR / PCR procedure
 - Processed 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
 - You might observe increasing PCR inhibition when your PCR mixture consist of >35% processed sample

06 WRITTEN PROTOCOL

Suggested thermal cycler 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 10 minutes (denaturation)
 - 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-RT
to ensure homogeneity

PCRopsis™ Reagent RVD-RT

Step 2



Mix 950 μ L Reagent
RVD-RT + 50 μ L
Activator to reservoir

Step 3



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

07 STEP-BY-STEP PROTOCOL WITH FIGURES

Step 4



Mix 20 μ L of sample to wells containing 20 μ L of reagent

PCRopsis™ Reagent RVD-RT

Step 5



Incubate mixture at room temperature for 10 minutes

Step 6



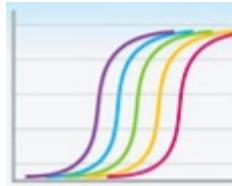
Add ~15 μ L PCR mix from your desired vendor to a new plate

07 STEP-BY-STEP PROTOCOL WITH FIGURES

Step 7



Step 8



Mix ~5 μ L of processed
sample mixture with your
PCR mix

Detect amplification of
target genes using your
desired PCR equipment

08 TROUBLESHOOTING & SUGGESTIONS

1. Reagent RVD-RT, with Activator, 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. Reagent RVD-RT, with Activator, may not thoroughly lyse bacteria at room temperature and a 10~15 minute heating step at 95°C may be required for optimal amplification of target genes.
3. For best results, use recently collected samples in compatible transport mediums that have been stored at ~4°C since collection.
4. 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.
5. Take care in maintaining the sterility of your Reagent RVD-RT stock after use.
6. Heating of activated Reagent RVD-RT + sample mixture may be required for some specimens if suboptimal results are observed.
7. It's recommended to use the processed activated Reagent RVD-RT + sample mixture for downstream applications within 4 hours to ensure optimal results.

09 CONTACT

Contact our research team if assistance with Reagent RVD-RT and / or Activator 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-RT

