



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 saliva or urine specimens or swab 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 sample (20 µL) with 1 volume of activated Reagent RVD-RT (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 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-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



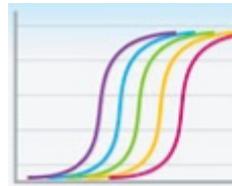
Add ~15 μ L PCR mix from your desired vendor to a new plate

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, saliva, and urine 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 processed sample may inhibit PCR for some applications.
4. Take care in maintaining the sterility of your Reagent RVD-RT stock after use.
5. Heating of activated Reagent RVD-RT + sample mixture may be required for some specimens if suboptimal results are observed.
6. 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.



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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v.20210914

PCRopsis™ Reagent RVD-RT

