



PCRopsis™ Reagent RVD with RVD Enhancer

(NOT FOR RESALE)

INTENDED USE (research use only)

PCRopsis™ Reagent RVD with RVD Enhancer is intended for nucleic acid extraction-free processing of liquid samples.

PRINCIPLES OF THE PROCEDURE

PCRopsis™ Reagent RVD is engineered to simultaneously bind a variety of reverse transcriptase quantitative polymerase chain reaction (RT-qPCR) / PCR inhibitors found in clinical samples, lyse microorganisms 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 nucleic acid extraction-free processing of liquid samples without centrifugations or other sample manipulations, which may introduce errors, contaminants and/or skew the representation of RNA fragments.

WARNINGS & PRECAUTIONS

For Research Use Only.

- Observe approved biohazard precautions and aseptic techniques to prevent contamination of the product. To be used only by adequately trained and qualified personnel.
- Directions should be read and followed carefully.
- Do not re-pack.
- Do not ingest.

Storage: This product is ready for use and no further preparation is necessary. The product should be transported and stored in its original container at 4–25°C until used. Do not overheat or freeze prior to use. Improper storage will result in a loss of efficacy. Do not use after expiration date, which is clearly printed on the label.

Product Deterioration: PCRopsis™ Reagent RVD with RVD Enhancer should not be used if (1) there is evidence of damage or contamination to the product, (2) there is evidence of leakage, (3) the color of the reagent has changed from clear-white hazy, (4) the expiration date has passed, or (5) there are other signs of deterioration.

PROCEDURES

Materials Provided: PCRopsis™ Reagent RVD with RVD Enhancer

Materials Required But Not Provided: Heating device (heating block or thermal cycler), thin walled tube (0.2 ~ 0.6 mL) or 96-well PCR plate, 15 or 50 mL conical, plate sealer, pipette tips and test sample

Test Procedure: Proper specimen collection, transport and storage is critical for successful nucleic acid amplification.

Compatible transport mediums:

- BD™ Universal Viral Transport System (UVT)
- Quest V-C-M Medium
- CitoSwab® VTM
- MedSchenker™ Smart Transport Medium
- SORFA Viral Transport Medium
- Mediums with recipes similar to BD™ UVT are expected to be compatible
- Copan ESwab™
- 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

Transport Mediums Not Recommended:

- Mediums containing guanidinium thiocyanate, alcohols, or other enzyme inhibitors

NOTE: Saliva samples processed with Reagent RVD with RVD Enhancer should be free of visual debris. This can be achieved by letting the saliva sample sit for over 5 minutes to allow debris to settle and collecting the mostly clear supernatant in the top phase. Saliva samples should be processed with PCRopsis™ Lysis Beads when working with bacteria, yeast / fungi, and spores.

NOTE: Urine samples processed with Reagent RVD with RVD Enhancer require the use of PCRopsis™ Support. Urine (10~25 mL) should be centrifuged at >1,400xg for 10 minutes to pellet cells and the supernatant removed, leaving <250 µL of residual urine to resuspend the pellet. This enriched cell sample is then processed with PCRopsis™ Lysis Beads.

NOTE: PCRopsis™ Lysis Beads are recommended when working with difficult to lyse bacteria, and yeast / fungi, and spores. Debris-free, liquid samples (~200 µL) are mixed with 0.25g of PCRopsis™ Lysis Beads in a U-bottom 2 mL tube, and vortexed on high for 5~10 minutes and then used as indicated below.

1. Thoroughly mix PCRopsis™ 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 Reagent RVD with RVD Enhancer (20 µL) with 1 volume of sample (20 µL) in a thin walled tube (0.2 ~ 0.6 mL) or 96-well PCR plate
 1. **For optimal results, the reagent needs to be added first to the tube before the sample is added.**
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 10~20 seconds before continuing
 1. Recommended heating times at 95°C:
 1. Mammalian: 5 minutes
 2. Viruses: 10~15 minutes

3. Bacteria / Fungi: 15~20 minutes
4. NOTE: heating for a longer period of time does not negatively affect results and may improve your gene amplification
2. Make sure the heating device has reached the desired temperature before applying sample.
3. You may need to increase the heating time if increasing the volume of sample and reagent past 100 µL of each
4. Sample heating can be performed using a controlled heating block or thermal cycler; however a device lid is highly recommended to minimize popping of tube caps or unpeeling of the plate sealer
5. Mix heated sample and use lysed / stabilized sample in your desired PCR procedure
 1. Lysed / stabilized sample should represent 15% ~ 30% of your final PCR 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

Quality Control: All lots of PCRopsis™ Reagent RVD with RVD Enhancer are tested for microbial contamination and the ability to amplify RNA / DNA without nucleic acid extraction. If aberrant quality control results are noted, results should not be reported.

AVAILABILITY – NOT FOR RESALE

Cat. #	Description
78336001	PCRopsis™ Reagent RVD with RVD Enhancer, 1 mL
78336025	PCRopsis™ Reagent RVD with RVD Enhancer, 25 mL
78336100	PCRopsis™ Reagent RVD with RVD Enhancer, 100 mL
783361000	PCRopsis™ Reagent RVD with RVD Enhancer, 1000 mL

MANUFACTURER

Entopsis, Inc., 7600 NW 69th Ave, Medley, FL 33166, USA info@entopsis.com

Glossary of Symbols Used



Research use only



Keep away from direct sunlight



Manufacturer's catalog number



Number of tests



Lot number



Consult instructions for use



Expiration date (year/month)



Sterile through aseptic techniques



Storage temperature



Manufacturer