



## **PCRopsis™ Activator**

( NOT FOR RESALE )

### **INTENDED USE (in vitro diagnostic use)**

PCRopsis™ Activator is intended in combination with PCRopsis™ technology to mediate room temperature Next Gen Direct PCR™.

### **PRINCIPLES OF THE PROCEDURE**

PCRopsis™ Activator is engineered to lyse viruses and cells, and stabilize nucleic acids at room temperature in a manner that's compatible with PCRopsis™ Next Generation Direct PCR™ reagents. This buffer uses a proprietary mixture of chemicals to achieve these tasks.

### **WARNINGS & PRECAUTIONS**

For in vitro Diagnostic Use.

- Observe approved biohazard precautions and aseptic techniques to prevent contamination of the product. To be used only by adequately trained and qualified personnel.
- Pathogenic microorganisms, including hepatitis viruses and Human Immunodeficiency Virus, may be present in clinical specimens. "Standard Precautions"<sup>1-4</sup> and institutional guidelines should be followed in handling all potentially bio-hazardous materials.
- Sterilize all biohazard waste including specimens, containers and mediums after their use.
- Directions should be read and followed carefully.
- Do not re-pack.
- The use of this product in association with a rapid diagnostic kit, diagnostic instrumentation or used in a manner not intended should be validated by the user.
- Do not ingest the reagent.
- Avoid skin contact with reagent since it contains sodium azide to prevent microbial growth.

**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 10–25°C. Do not overheat. Do not freeze prior to use. Storage at ~4°C may result in an insoluble precipitate that may negatively affect the functionality of this product. Improper storage will result in a loss of efficacy. Do not use after expiration date, which is clearly printed on the label.

**Product Deterioration:** Product 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 yellowish-clear, (4) the expiration date has passed, or (5) there are other signs of deterioration.

### **PROCEDURES**

**Materials Provided:** PCRopsis™ Activator



**Materials Required But Not Provided:** PCR*opsis*™ Reagent RVD-RT, thermal cycler, thin walled tube (0.2 ~ 0.6 mL) or 96-well PCR plate, plate sealer, pipette tips, PCR kit with primers / probes, and test sample

**Test Procedure:** Proper specimen collection, transport and storage is critical for successful nucleic acid amplification. For specific guidance regarding specimen collection procedures, consult published reference manuals.<sup>5-11</sup> Clinical specimens should be collected as soon as possible after the clinical onset of disease. Highest viral titers are present during the acute illness.

**Compatible transport mediums:**

- BD™ Universal Viral Transport System (UVT) - preferred
- 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 or alcohols
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
  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 diluted sample at room temperature (~25°C) for 10 minutes
  5. Immediately mix processed sample and use in your desired RT-qPCR / PCR procedure
    1. 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
    2. You might observe increasing PCR inhibition when your PCR mixture consist of >35% processed sample



### 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 10 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

**Quality Control:** All lots of PCRopsis™ Activator are tested for microbial contamination and the ability to improve the amplification of bacterial targets from urine samples. If aberrant quality control results are noted, patient results should not be reported.

### **RESULTS**

Results obtained will partially depend on proper and adequate specimen collection, transport and processing in the laboratory.

### **LIMITATIONS OF THE PROCEDURE**

- Performance characteristics of PCRopsis™ Activator, in combination with PCRopsis™ Reagent RVD-RT, were validated using SARS-CoV-2 virus and human epithelial cells in numerous transport mediums and 100% saliva or urine specimens through PCR / RT-qPCR. The use of alternative microorganisms, transport mediums, gene targets and / or detection methods may affect the performance of the product.
- Repeated freezing and thawing of test specimens may reduce the detection of desired gene targets.
- Activated Reagent RVD-RT should be used within 24 hours if stored at room temperature in the dark
- The activated Reagent RVD-RT – sample mixture must be used within 4 hours for downstream PCR applications after the 10-minute incubation at room temperature to ensure optimal results.
- Follow recommended guidelines for specimen collection, transport and storage as this may affect the ability to amplify gene targets.

### **PERFORMANCE CHARACTERISTICS**

The performance of activated PCRopsis™ Reagent RVD-RT was compared to traditional RNA extraction methods (e.g., Qiagen's QIAamp Viral RNA Kit) from the same samples. These studies used SARS-CoV-2 spiked into transport mediums, spiked samples processed using both methods and RT-qPCR was performed using Integrated DNA Technologies (IDT) qPCR probe assay and Promega GoTaq® Probe 1-Step RT-qPCR System. Observed Ct values between both methods were usually within a few Ct of each other.



## AVAILABILITY – NOT FOR RESALE

Cat. #	Description
228001	PCRopsis™ Activator, 1.5 mL











## MANUFACTURER

Entopsis, Inc., 7600 NW 69th Ave, Medley, FL 33166, USA [info@entopsis.com](mailto:info@entopsis.com)

## REFERENCES

1. Clinical and Laboratory Standards Institute. 2005. Approved Guideline M29-A3. Protection of laboratory workers from occupationally acquired infections, 3rd ed. CLSI, Wayne, Pa.
2. Garner, J.S. 1996. Hospital Infection Control Practices Advisory Committee, U.S. Department of Health and Human Services, Centers for Disease Control and Prevention. Guideline for isolation precautions in hospitals. *Infect. Control Hospital Epidemiol.* 17: 53-80.
3. U.S. Department of Health and Human Services. 1999. Biosafety in microbiological and biomedical laboratories, HHS Publication (CDC), 4th ed. U.S. Government Printing Office, Washington, D.C.
4. Directive 2000/54/EC of the European Parliament and of the Council of 18 September 2000 on the protection of workers from risks related to exposure to biological agents at work (seventh individual directive within the meaning of Article 16(1) of Directive 89/391/EEC). *Official Journal L262*, 17/10/2000, p. 0021-0045.
5. Murray, P.R., E.J. Baron, J.H. Jorgensen, M.A. Pfaller, and R.H. Tenover. 2003. *Manual of clinical microbiology*. 8th ed. ASM, Washington, D.C.
6. Gleaves, C.A., R.L. Hodinka, S.L.G. Johnston, and E.M. Swierkosz. 1994. *Cumitech 15A. Laboratory diagnosis of viral infections*. ASM, Washington, DC.
7. Forbes, B.A., D.F. Sahm, and A.S. Weissfeld. 2002. *Bailey and Scott's diagnostic microbiology*. 11th ed. Mosby, St. Louis, MO.
8. Wardford, A., M. Chernesky, and E. M. Peterson. 1999. *Cumitech 19A, Laboratory diagnosis of Chlamydia trachomatis infections*. ASM, Washington, DC.
9. Miller, J. M. 1999. *A guide to specimen management in clinical microbiology*, 2nd ed. ASM, Washington, DC.
10. Isenberg, H. D., 2004. *Clinical microbiology procedures handbook*, 2nd ed. ASM, Washington, DC.
11. Isenberg, H.D., 1998. *Essential procedures for clinical microbiology*. Chapter 14.12, Page 787. Packaging and shipping infectious substances.

## Glossary of Symbols Used

	In vitro diagnostic use		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