



PCRopsis™ Blood Kit (beta version)

(NOT FOR RESALE)

INTENDED USE (Research Use Only)

PCRopsis™ Blood Kit is intended as an extraction-less procedure performed on properly collected and transported plasma and serum specimens in compatible transport tubes before nucleic acid amplification of RNA or DNA.

PRINCIPLES OF THE PROCEDURE

PCRopsis™ Blood Kit is engineered to simultaneously bind a variety of reverse transcriptase quantitative polymerase chain reaction (RT-qPCR) / PCR inhibitors found in plasma and serum 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 proprietary molecules to achieve these tasks. The PCRopsis™ Blood Kit allows for extraction-free amplification of RNA / DNA without performing nucleic acid extraction / isolation.

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.
- Pathogenic microorganisms, including hepatitis viruses and Human Immunodeficiency Virus, may be present in clinical specimens. "Standard Precautions"¹⁻⁴ 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 reagents or beads.
- Ensure individual kit components are not expired
- 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 until used. Do not overheat. Do not 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™ Blood Kit 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 Blood, PCRopsis™ Activator, PCRopsis™ BPS

Materials Required But Not Provided: Heating device (heating block or thermal cycler), centrifuge, vortexer, thin walled tube (0.2 ~ 0.6 mL) or 96-well PCR plate, plate sealer, pipette tips 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.⁵⁻¹¹ 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 Blood transport tubes:

- EDTA blood collection tubes
- Serum blood collection tubes

Blood Sample:

- Plasma
- Serum

Prepare Activated Reagent Blood:

1. Heat PCRopsis™ BPS for 10~15 minutes at 95°C in a heating device
 - a. An aliquot of PCRopsis™ BPS should be added to a PCR tube and then heated
 2. Immediately add 10 µL of heated PCRopsis™ BPS to 940 µL PCRopsis™ Reagent Blood and immediately mix thoroughly by vortexing the mixture
 - a. The time between PCRopsis™ BPS is removed from the heating device and added to PCRopsis™ Reagent Blood should be less than 10 seconds
 - b. Thoroughly mix PCRopsis™ Reagent Blood to ensure homogeneity before using, but avoid creating bubbles unnecessarily
 - c. The volumes of each reagent can be scaled up using the same ratio
 3. Add 50 µL PCRopsis™ Activator to the Reagent Blood – BPS mixture, and mix thoroughly
 - a. This mixture is called Activated Reagent Blood from here onward
 - b. Activated Reagent Blood is stable for 4 hours when stored at room temperature and 24 hours when stored at 4°C
-
1. Mix 1 volume of Activated Reagent Blood (20 µL) with 1 volume of blood sample (20 µL) in a thin walled tube (0.2 ~ 0.6 mL) or 96-well PCR plate
 - 1. For optimal results, the activated reagent needs to be added first to the tube before the sample is added.**
 2. Pipette up & down to ensure complete mixing and then cap tube or apply plate sealer to plate to prevent evaporation
 3. Let the diluted sample sit at room temperature for 10 minutes
 4. Mix the processed sample and use lysed / stabilized sample in your desired PCR procedure



1. Lysed / stabilized sample should represent 10% ~ 15% of your final PCR mixture (i.e., 2~3 μL sample into a total volume of 20 μL) depending on the polymerase used
2. You might observe increasing PCR inhibition and / or reduced fluorescent signal when your PCR mixture consist of >20% processed sample

Suggested thermocycler parameters for RT-PCR / PCR:

1. Reverse transcription:
 - a. 45°C for 15 minutes
 - b. 95°C for 10 minutes
2. PCR amplification (~45 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

Quality Control: All lots of PCR*opsis*™ Reagent Blood, PCR*opsis*™ Activator, and PCR*opsis*™ BPS are tested for microbial contamination and the ability to amplify RNA / DNA without nucleic acid extraction. 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. The use of PCR*opsis*™ Blood Kit with incompatible blood collection tubes, alternative sample types, and with noticeable microbial growth (i.e., contamination) may result in unreliable results.

LIMITATIONS OF THE PROCEDURE

- The performance characteristics of the PCR*opsis*™ Blood Kit were validated using SARS-CoV-2 spiked into plasma as a model system. The use of alternative microorganisms, transport mediums, gene targets and / or detection methods may affect the performance of the product.
- RT-qPCR / PCR cycle thresholds (Ct) should be set higher than when extracted RNA / DNA is utilized for amplification (e.g., 40~45 cycles).
- Repeated freezing and thawing of test specimens may reduce the detection of desired gene targets.
- Unreliable results may be observed if PCR*opsis*™ Blood Kit reagents are stored improperly. Activated Reagent Blood, the mixture of the 3 components, is stable for 4 hours when stored at room temperature and 24 hours when stored at 4°C.
- Follow recommended guidelines for specimen collection, transport and storage as this may affect the ability to amplify gene targets.



PERFORMANCE CHARACTERISTICS

The performance of the PCR*opsis*™ Blood Kit will be compared to alternative approaches in the future.

AVAILABILITY – NOT FOR RESALE

Cat. #	Description
25500100	PCR <i>opsis</i> ™ Blood Kit (100 tests)
25501000	PCR <i>opsis</i> ™ Blood Kit (1,000 tests)
25510000	PCR <i>opsis</i> ™ Blood Kit (10,000 tests)











MANUFACTURER

Entopsis, Inc., 7600 NW 69th Ave, Medley, FL 33166, USA 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