



PCRopsis™ Support

(NOT FOR RESALE)

INTENDED USE (in vitro diagnostic use)

PCRopsis™ Support is intended to improve the functionality of Next Generation Direct PCR™ reagents for the detection of microorganisms from urine.

PRINCIPLES OF THE PROCEDURE

PCRopsis™ Support is engineered to improve the accessibility of specimen RNA / DNA from select samples, such as urine, and thereby improve PCR amplification. This buffer uses a mixture of salts, peptides, and proteins to improve access of sample components to active complexes found in Next Generation Direct PCR™ reagents.

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"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 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 4–25°C until used. Do not overheat. Do not incubate 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™ Support 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™ Support



Materials Required But Not Provided: PCRopsis™ Reagent RVD with RVD Enhancer (see product IFU), thermal cycler, heating device (heating block or thermal cycler), 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.

Specimen: undiluted urine

1. Centrifuge 1.5~25 mL of urine at >1,400xg for 10 minutes in a 15 mL or 50 mL conical
 1. Higher volumes of centrifuged urine is expected to result in higher sensitivity
2. Remove supernatant and leave <250 µL of residual urine
3. Resuspend cell pellet using residual urine

Recommended for difficult to lyse bacteria and yeast / fungi:

1. Add 200 µL of sample to a vial or well containing roughly 0.3 grams PCRopsis™ Lysis Beads (see product IFU)
 2. Cap tube or place a plate sealer on the deep well plate
 3. Vortex on high for ~5 minutes to lyse microorganisms
4. Thoroughly mix 2 µL of PCRopsis™ Support with 1 mL PCRopsis™ Reagent RVD with RVD Enhancer (called **RVD-Support solution** from here on)
 1. This mixture is stable for ~24 hours at room temperature
 2. If poor results are observed, then add 10 ~ 20 µL of PCRopsis™ Support per 1 mL PCRopsis™ Reagent RVD with RVD Enhancer
 5. Mix 20 µL RVD-Support solution with 20 µL of the resuspended urine pellet in a thin-walled tube (0.2 ~ 0.6 mL)
 1. **For optimal results, the reagent needs to be added first to the tube before the sample is added.**
 2. Ratio of sample to PCRopsis™ reagent will remain 1:1, but volume can be increased if needed (example: 30 µL : 30 µL and so forth)
 6. Pipette up & down to ensure complete mixing and then cap tube or apply plate sealer to plate to prevent evaporation
 7. Heat diluted sample at 95°C for 10 ~ 15 minutes and let cool at room temperature for ~10 seconds before continuing
 1. Heating for a slightly longer period (extra 2~5 minutes) of time does not negatively affect results
 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



8. 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 μ L sample into a total volume of 20 μ L) 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 PCR*opsis*[™] Support 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 PCR*opsis*[™] Support, in combination with PCR*opsis*[™] Reagent RVD with RVD Enhancer, were validated using human urine spiked with bacteria. Bacterial DNA was amplified through PCR. The use of alternative samples, reagents 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.
- The RVD-Support solution – sample mixture must be used within 4 hours for downstream PCR applications after the 10-minute incubation at 95°C 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 PCR*opsis*[™] Support in combination with PCR*opsis*[™] Reagent RVD with RVD Enhancer was confirmed using human urine spiked with bacteria as the test sample. The test sample was processed as recommended and bacteria-specific 16S rRNA gene was amplified through PCR.

AVAILABILITY – NOT FOR RESALE

Cat. #	Description
787002	PCR <i>opsis</i> [™] Support, 0.25 mL
787001	PCR <i>opsis</i> [™] Support, 1 mL











MANUFACTURER

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REFERENCES

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Glossary of Symbols Used

 IVD	In vitro diagnostic use		Keep away from direct sunlight
 REF	Manufacturer's catalog number		Number of tests
 LOT	Lot number		Consult instructions for use
	Expiration date (year/month)		Sterile through aseptic techniques
	Storage temperature		Manufacturer