PCR*opsis*™ Reagent RVD with RVD Enhancer

(NOT FOR RESALE)

INTENDED USE (in vitro diagnostic use)

PCR*opsis*™ Reagent RVD with RVD Enhancer 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.

PRINCIPLES OF THE PROCEDURE

PCR*opsis*™ 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 extraction-free amplification of RNA / DNA without performing nucleic acid extraction, centrifugations or other sample manipulations, which may introduce errors, contaminants and/or skew the representation of RNA fragments.

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: PCR*opsis*™ 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: PCR*opsis*™ 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, 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 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, 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.

NOTE: **Urine samples** processed with Reagent RVD with RVD Enhancer require the use of PCR*opsis*™ Support. Urine (1.5~25 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.

NOTE: PCR*opsis*™ Lysis Beads are recommended when amplifying gene targets from difficult to lyse bacteria and yeast / fungi.

1. Thoroughly mix PCR*opsis*™ 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 minutes
        3. Bacteria: 15 minutes
        4. NOTE: heating for a longer period of time does not negatively affect results and may improve your detection limit
    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

    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

Quality Control: All lots of PCR*opsis*™ 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, 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*™ Reagent RVD with RVD Enhancer with incompatible transport mediums or mediums with noticeable microbial growth (i.e., contamination) may result in unreliable results.

LIMITATIONS OF THE PROCEDURE

- Performance characteristics of PCR*opsis*™ Reagent RVD with RVD Enhancer were validated using SARS-CoV-2, S. *aureus* and P. *aeurginos* through RT-qPCR or qPCR in select transport mediums. The use of alternative microorganisms, transport mediums, gene targets and / or detection methods may affect the performance of the product.
- RT-qPCR cycle thresholds (Ct) are often set higher than when extracted RNA / DNA is utilized (e.g., 40~45 cycles).
- Repeated freezing and thawing of test specimens may reduce the detection of desired gene targets.
- 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*™ Reagent RVD with RVD Enhancer 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
78336001	PCR*opsis*™ Reagent RVD with RVD Enhancer, 1 mL (for validation purposes only)
78336025	PCR*opsis*™ Reagent RVD with RVD Enhancer, 25 mL
78336100	PCR*opsis*™ Reagent RVD with RVD Enhancer, 100 mL
783361000	PCR*opsis*™ Reagent RVD with RVD Enhancer, 1000 mL

MANUFACTURER

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REFERENCES

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Glossary of Symbols Used