PCR*opsis*™ Reagent RVD-RT

(not for resale)

INTENDED USE (in vitro diagnostic use)

PCR*opsis*™ Reagent RVD-RT is intended for extraction-free amplification of RNA or DNA from properly collected and transported viral specimens in compatible transport mediums at room temperature.

PRINCIPLES OF THE PROCEDURE

PCR*opsis*™ Reagent RVD-RT, in combination with PCR*opsis*™ Activator, is engineered to simultaneously bind a variety of reverse transcriptase quantitative polymerase chain reaction (RT-qPCR) / PCR inhibitors found in clinical samples, lyse specimens 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, RVD Enhancer, and sodium azide to achieve these tasks. Reagent RVD-RT 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. Reagent RVD-RT should be transported and stored in its original container at 4–25˚C, while PCR*opsis*™ Activator should be transported and stored in its original container at 10–25˚C. Do not store the mixture of Reagent RVD-RT with Activator for more than 4 hours at room temperature or more than 24 hours at 4˚C. Do not overheat. Do not freeze prior to use. Bring product to room temperature if ice crystals are observed. 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 clear-white hazy, (4) the expiration date has passed, or (5) there are other signs of deterioration.

PROCEDURES
Materials Provided: PCR*opsis*™ Reagent RVD-RT and PCR*opsis*™ Activator

Materials Required But Not Provided: Thermal cycler, tube (0.1 ~ 0.5 mL) or 96-well PCR plate, plate sealer, pipette tips, PCR kit with primers / probe, and test sample

Test Procedure: Proper specimen collection, transport and storage are 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-RT 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.

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 ~4 hours at room temperature and ~24 hours at 4˚C
3. Mix 1 volume of activated Reagent RVD-RT (20 µL) with 1 volume of sample (20 µL) in a sterile tube (0.1 ~ 0.5 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.**

4. Thoroughly pipette up & down to ensure complete mixing and then cap tube or apply plate sealer to plate to prevent evaporation
5. Incubate sample mixture at room temperature (~25˚C) for 10 minutes
    1. The sample mixture can be heating at 95˚C for 10 minutes when processing difficult to lyse microorganisms or for select applications
    2. Processed sample should be used within 4 hours when stored at room temperature
6. 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 minute denaturation
            - 95˚C for 5 seconds
            - 55˚C for 15 seconds
            - 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-RT 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-RT 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 activated Reagent RVD-RT were validated using SARS-CoV-2 virus and human epithelial cells in numerous transport mediums and 100% saliva 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.

- The product may not completely lyse all bacteria at room temperature and thereby may result in poor amplification of these specimens. Heating the reagent-sample mixture for 10~15 minutes at 95˚C improves bacterial lysis.
- Repeated freezing and thawing of test specimens may reduce the detection of desired gene targets.
- Activated Reagent RVD-RT should be used within 4 hours if stored at room temperature or within 24 hours if stored at 4˚C 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 PCR*opsis*™ 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
78378001	PCR*opsis*™ Reagent RVD-RT, 1 mL (for validation purposes only)
78378025	PCR*opsis*™ Reagent RVD-RT, 25 mL
78378100	PCR*opsis*™ Reagent RVD-RT, 100 mL
783781000	PCR*opsis*™ Reagent RVD-RT, 1000 mL

MANUFACTURER

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REFERENCES

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Glossary of Symbols Used