PCR*opsis*™ Reagent SRVD

(NOT FOR RESALE)

INTENDED USE (in vitro diagnostic use)

PCR*opsis*™ Reagent SRVD is intended for extraction-free amplification of RNA or DNA from saliva samples.

PRINCIPLES OF THE PROCEDURE

PCR*opsis*™ Reagent SRVD is engineered to simultaneously bind a variety of reverse transcriptase quantitative polymerase chain reaction (RT-qPCR) / PCR inhibitors found in saliva 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 peptide, salts, stabilizers, buffers, RVD Enhancer, and sodium azide to achieve these tasks. Reagent SRVD 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 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 SRVD 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 SRVD

Materials Required But Not Provided: Heating device (heating block or thermal cycler), 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 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.

Test Sample:
- 100% saliva
- the compatibility of saliva transport mediums needs to be confirmed

NOTE: Saliva samples processed with Reagent SRVD 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.

Transport Mediums Not Recommended:
- Mediums containing guanidinium thiocyanate, alcohols, or other enzyme inhibitors

1. Thoroughly mix PCR*opsis*™ Reagent SRVD to ensure homogeneity, but avoid creating bubbles unnecessarily
    1. Reagent SRVD has a hazy, white color when homogenized and normal settlement occurs if not regularly mixed
2. Mix 1 volume of sample (20 µL) with 1 volume of Reagent SRVD (20 µL) in a thin walled tube (0.2 ~ 0.6 mL) or 96-well PCR plate
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
    2. Make sure the heating device has reached the desired temperature before applying sample.
    3. Sample heating can be performed using a controlled heating block or thermal cycler
5. Mix heated sample and use lysed / stabilized sample in your desired PCR / RT-qPCR procedure
    1. Lysed 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

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 SRVD are tested for microbial contamination and the ability to amplify RNA / DNA without 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, selection of gene target for amplification, and processing in the laboratory. The use of PCR*opsis*™ Reagent SRVD 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 SRVD were validated using SARS-CoV-2, S. *aureus* and P. *aeurginos* in human saliva. 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 SRVD was compared to traditional RNA extraction methods from the same samples. These studies used SARS-CoV-2 or bacteria spiked into saliva, spiked samples processed using both methods and RT-qPCR was performed using IDT qPCR probe assay and Promega GoTaq® Probe 1-Step RT-qPCR System. Observed Ct values between both methods were usually within 5 Ct of each other, where samples processed with Reagent SRVD were usually greater than when the same sample was processed by RNA extraction.

AVAILABILITY – NOT FOR RESALE

Cat. #	Description
7783001	PCR*opsis*™ Reagent SRVD, 1 mL (for validation purposes only)
7783025	PCR*opsis*™ Reagent SRVD, 25 mL
7783100	PCR*opsis*™ Reagent SRVD, 100 mL
77831000	PCR*opsis*™ Reagent SRVD, 1000 mL

MANUFACTURER

Entopsis, Inc., 7600 NW 69th Ave, Medley, FL 33166, USA info@entopsis.com

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 A	Sterile through aseptic techniques
	Storage temperature		Manufacturer