PCR*opsis*™ Reagent SRVD

For In Vitro Diagnostic Use

REF #: 7783025, 7783100, 77831000

REF #: 7783001 (for validation purposes only)

Store at room temperature

INTENDED USE

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

01 INTRODUCTION

PCR*opsis*™ Reagent SRVD is engineered to simultaneously bind a variety of reverse transcriptase quantitative polymerase chain reaction (RT-qPCR) and PCR inhibitors found in saliva specimens, 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 SRVD allows for extraction-free amplification of RNA / DNA from saliva specimens without performing nucleic acid isolation, centrifugations or other sample manipulations, which may introduce errors, contaminants and/or skew the representation of RNA fragments.

02 PRODUCT SIZE

Catalog Number	Volume
7783001	1 mL
7783025	25 mL
7783100	100 mL
77831000	1000 mL

03 STORAGE & STABILITY

PCR*opsis*™ Reagent SRVD is shipped and stored at room temperature. The recommended storage temperature is: 4˚C ~ 25˚C

04 TRANSPORT MEDIUM COMPATIBILITY

RECOMMENDED:

- no transport medium
- Universal Transport Mediums
- Viral Transport Mediums
- 10 mM Tris pH 8 + antibiotics

NOT RECOMMENDED:

- Mediums containing guanidinium thiocyanate

NOTE: the user must confirm the compatibility of Reagent SRVD with desired saliva samples

05 OVERVIEW OF PROTOCOL

Reagent SRVD

Mix 20 µL Reagent SRVD + 20 µL Sample / well

Heat Reagent SRVD + Sample mixture to 95˚C for recommended time

Use processed sample into your desired RT-qPCR / PCR mixture (sample = <30% of total volume)

Saliva Sample

NOTE: samples can be heated in a thermal cycler or heating block

Recommended heating times:
- Mammalian: 5 minutes
- Viruses: 10 minutes
- Bacteria: 15 minutes

NOTE: heating for a longer period of time does not negatively affect results

06 WRITTEN PROTOCOL

1. Thoroughly mix 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 saliva 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 seconds before continuing
    1. Recommended heating times at 95˚C:
        1. Mammalian: 5 minutes
        2. Viruses: 10 minutes
        3. Bacteria: 15 minutes

        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 RT-qPCR / PCR 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

06 WRITTEN PROTOCOL

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

07 STEP-BY-STEP PROTOCOL WITH FIGURES

Step 1

Gently invert Reagent SRVD to ensure homogeneity

Step 2

Add Reagent SRVD to reservoir

Step 3

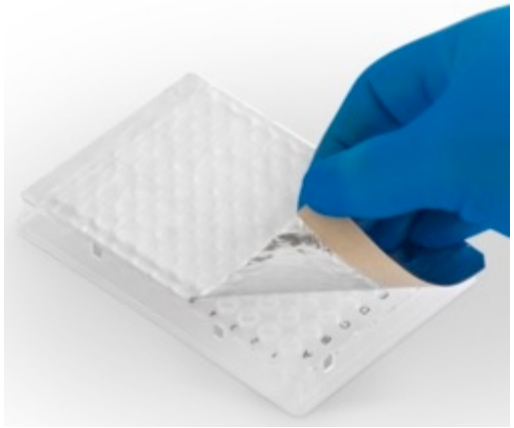
Add 20 µL of Reagent SRVD to wells in a 96-well PCR plate

07 STEP-BY-STEP PROTOCOL WITH FIGURES

Step 4

Mix 20 µL of sample to wells containing 20 µL of Reagent SRVD

Step 5



Seal 96-well plate with a plate sealer to prevent evaporation

Step 6

Pre-heated thermal cycler or heating block before applying plate

<u>Recommended heating times</u>:

- Mammalian: 5 minutes
- Viruses: 10 minutes
- Bacteria: 15 minutes

<u>NOTE</u>: heating for a longer period of time does not negatively affect results

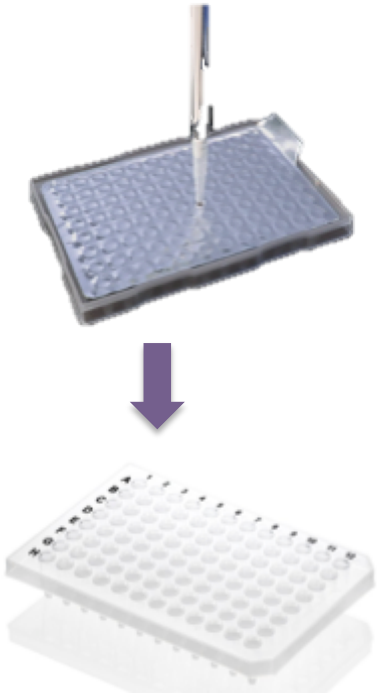
Heat Reagent SRVD + sample mixture at 95˚C for recommended time

07 STEP-BY-STEP PROTOCOL WITH FIGURES

Step 7

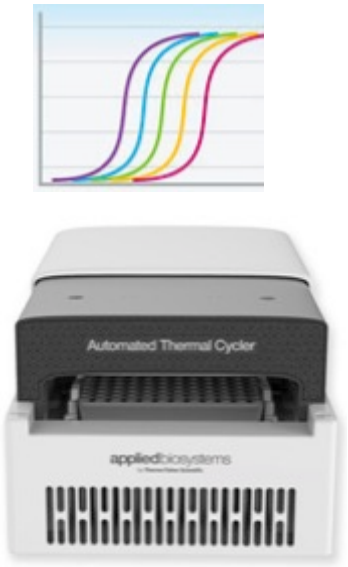
Add 15 µL RT-qPCR mix from your desired vendor to a new plate

Step 8



Mix 5 µL of heated sample + Reagent SRVD mixture with your RT-qPCR mix

Step 9



Detect amplification of target genes using your desired qPCR equipment

08 TROUBLESHOOTING & SUGGESTIONS

1. Reagent SRVD is optimized for the amplification of gene targets from saliva specimens and may not be applicable for other applications or mediums.
2. For best results, use recently collected samples in compatible transport mediums that have been stored at ~4˚C since collection.
3. Samples are diluted 50% with Reagent SRVD. As such, you should expect to observe slightly higher Ct's compared to nucleic acid extracted samples.
4. Ensure that the processed sample consist of <30% of the total PCR mixture, since high concentrations of the processed sample may inhibit PCR for some applications.
5. Ct cut-offs for assays should be increased, often times to 45 cycles.
6. Take care in maintaining the sterility of your Reagent SRVD stock after use.
7. Heat Reagent SRVD / sample mixture for a few minutes longer if you observe suboptimal lysis.
8. It's recommended to use the heated Reagent SRVD + sample mixture for downstream applications within a day, although samples may be stable for months at 4˚C or -20˚C.

09 CONTACT

Contact our research team if assistance with Reagent SRVD is necessary (**info@entopsis.com**). We will try our best to assist with non-intended applications of this product or direct you to alternative products. Any business related questions should be directed to: **Sales@PCRopsis.com**.

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