PCR*opsis*™ Reagent Buccal

For In Vitro Diagnostic Use

REF #: 282025, 282100, 282500 & 2821000

REF #: 282001 (for validation purposes only)

Store at room temperature

INTENDED USE

PCR*opsis*™ Reagent Buccal is intended for extraction-free PCR amplification of RNA and DNA from human buccal cells on synthetic swabs without the need for transport mediums.

01 INTRODUCTION

PCR*opsis*™ Reagent Buccal is engineered to simultaneously elute material from swabs, bind a variety of reverse transcriptase quantitative polymerase chain reaction (RT-qPCR) / PCR inhibitors found in buccal specimens, lyse specimens, and stabilize nucleic acids in a manner that's compatible with PCR. The product consists of a proprietary mixture of peptides, salts, stabilizers, buffers and sodium azide to achieve this task. Reagent Buccal allows for extraction-free amplification of nucleic acids without performing extractions, centrifugations or other sample manipulations, which may introduce errors, contaminants and/or skew the representation of RNA fragments.

02 PRODUCT SIZE

Catalog Number	Volume
282001	1 mL
282025	25 mL
282100	100 mL
282500	500 mL
2821000	1000 mL

03 STORAGE & STABILITY

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

04 OVERVIEW OF PROTOCOL

1) Add 100 ~ 200 µL Reagent Buccal to tube with test swab
2) Vortex for ~30 seconds, 3 times, to elute specimen off swab

Reagent Buccal

Add ~50 µL of eluted sample to a thin-walled tube or plate

Heat eluted sample to 95°C for 10 minutes

Use processed sample into your desired RT-qPCR / PCR mixture

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

05 WRITTEN PROTOCOL

1. Thoroughly mix Reagent Buccal to ensure homogeneity, but avoid creating bubbles unnecessarily
    1. Reagent Buccal has a hazy, white color when homogenized and normal settlement occurs if not regularly mixed
2. Elute material from swab:
    1. Add 100 ~ 200 µL of Reagent Buccal to transport tube with swab
    2. Make sure the swab is at least partially submerged into Reagent Buccal
    3. Vortex for 30 seconds, 3 times, to elute sample
    4. Press the swab against the walls of the tube to release reagent with cells
3. Specimen lysis & nucleic acid stabilization:
    1. Transfer 50 ~ 100 µL of eluted sample into a thin-walled PCR tube / plate and then cap tube or apply plate sealer to plate to prevent evaporation
    2. Heat at 95˚C for 10 minutes
    3. Let cool at room temperature for ~10 seconds before continuing

05 WRITTEN PROTOCOL

4. Pipette up & down to ensure complete mixing
5. Use processed sample in your desired PCR procedure
    1. Processed sample should represent 10%~30% of your final PCR mixture (i.e., 2~6 µL sample into a total volume of 20 µL)
    2. You might observe increasing PCR inhibition when your PCR mixture consist of >35% processed sample

05 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

06 STEP-BY-STEP PROTOCOL WITH FIGURES

Step 1

Gently invert Reagent Buccal to ensure homogeneity

Step 2

Add ~100 µL Reagent Buccal to transport tube containing test swab

Step 3

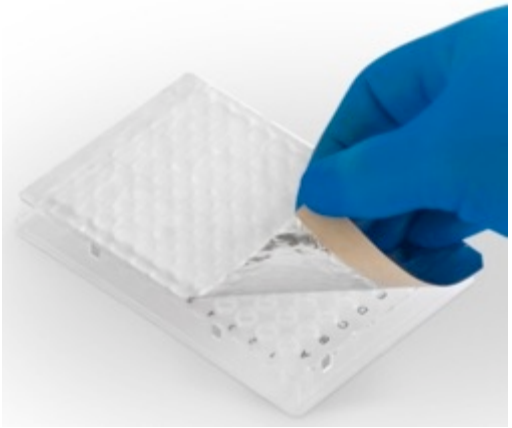
Vortex tube with swab and Reagent Buccal to elute specimen off swab

06 STEP-BY-STEP PROTOCOL WITH FIGURES

Step 4

Transfer ~50 µL of eluted sample to thin-walled plate or tube

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 or tube

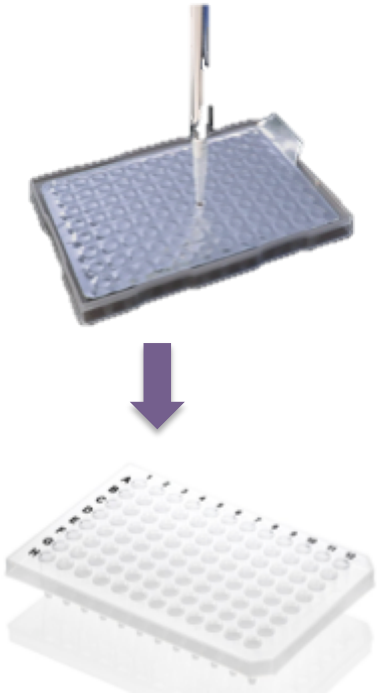
Heat eluted mixture at 95˚C for 10 minutes

06 STEP-BY-STEP PROTOCOL WITH FIGURES

Step 7

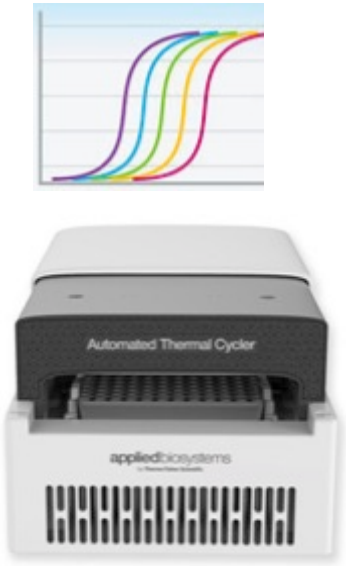
Add 15 µL PCR mix from your desired vendor to a new plate

Step 8



Mix 5 µL of heated, eluted sample with your PCR mix

Step 9



Detect amplification of target genes using your desired equipment

07 TROUBLESHOOTING & SUGGESTIONS

1. Reagent Buccal is optimized for the amplification of gene targets from human buccal cells on swabs without transport medium and may not be applicable for other applications.
2. For best results, use recently collected samples that have been properly stored since collection.
3. A complete validation study is warranted when using alternative swabs and lower volumes of Reagent Buccal to improve assay sensitivity.
4. Ensure that the processed sample consist of <30% of the total PCR mixture, since high concentrations of processed sample may inhibit PCR for some applications.
5. Take care in maintaining the sterility of your Reagent Buccal stock after use.
6. Heat Reagent Buccal + sample mixture for a few minutes longer if you observe suboptimal lysis.
7. It's recommended to use the heated Reagent Buccal + sample mixture for downstream applications within a day, although storage at 4˚C ~ -20˚C may be acceptable for many gene targets.

08 CONTACT

Contact our research team if assistance with Reagent Buccal 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**.

7600 NW 69th Ave, Medley, FL 33166
www.PCRopsis.com

info@entopsis.com
sales@PCRopsis.com

Toll-free 1-888-407-5070

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