



PCRopsis™ Reagent Buccal

(Not for Resale)

INTENDED USE (in vitro diagnostic use)

PCRopsis™ Reagent Buccal is intended for nucleic acid extraction-free processing of human buccal cells on synthetic swabs without the need for transport mediums.

PRINCIPLES OF THE PROCEDURE

PCRopsis™ 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.

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.
- 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: PCRopsis™ Reagent Buccal 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: PCRopsis™ Reagent Buccal

Materials Required But Not Provided: Thermal cycler, heating device (heating block or thermal cycler), synthetic buccal swab, transport tubes, thin walled tube (0.2 ~ 0.6 mL) or 96-well PCR plate, plate sealer, pipette tips, PCR kit, and sample

Test Procedure: Collect test specimen by rubbing once or twice the inner cheek region with a buccal swab. Proper specimen collection, transport and storage is critical for successful nucleic acid amplification.

Recommended swabs: synthetic buccal swabs (nylon, rayon, dacron, polyester) with aluminum or plastic shafts

Material to be tested: specimen-containing swab properly transported in a sterile tube without transport medium

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 the transport tube with the 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
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

Suggested thermocycler parameters for RT-PCR / PCR:

1. Reverse transcription:
 - a. 45°C for 15 minutes (extend to 30 minutes if suboptimal results observed)
 - 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:

- When amplifying RNA, 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 PCRopsis™ Reagent Buccal are tested for microbial contamination and the ability to amplify human DNA from cheek cells without nucleic acid extraction. If aberrant quality control results are noted, results should not be reported.

RESULTS

Results obtained will partially depend on proper and adequate specimen collection, transport and processing in the laboratory. PCRopsis™ Reagent Buccal may result in unreliable results when used beyond the intended use.

LIMITATIONS OF THE PROCEDURE

- Performance characteristics of PCRopsis™ Reagent Buccal were validated using dried human cheek cells on buccal swabs and PCR amplification of GAPDH. The use of alternative cells, swabs, gene targets and / or detection methods may affect the performance of the product.
- Improper transport and storage of test swabs may reduce the detection of desired gene targets.
- Buccal transport mediums with alcohol or guanidine thiocyanate are not compatible with this product.
- Follow recommended guidelines for specimen collection, transport and storage as this may affect the ability to amplify gene targets.

PERFORMANCE CHARACTERISTICS

The performance of PCRopsis™ Reagent Buccal was determined by diluting reagent-processed buccal specimens over one million fold and then performing PCR using primers specific for GAPDH. These studies used human cheek cells absorbed and dried onto buccal swabs and qPCR was performed using IDT primers / probe and PCRopsis™ 5x PCR Master Mix for 45 cycles.











AVAILABILITY – NOT FOR RESALE

Cat. #	Description
282001	PCRopsis™ Reagent Buccal, 1 mL
282025	PCRopsis™ Reagent Buccal, 25 mL
282100	PCRopsis™ Reagent Buccal, 100 mL
2821000	PCRopsis™ Reagent Buccal, 1,000 mL

MANUFACTURER

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

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 through aseptic techniques
	Storage temperature		Manufacturer