



PCRopsis™ Reagent 123 (beta) (NOT FOR RESALE)

INTENDED USE (research use only)

PCRopsis™ Reagent 123 is intended for nucleic acid extraction-free processing of diverse sample types.

PRINCIPLES OF THE PROCEDURE

PCRopsis™ Reagent 123 is engineered to simultaneously bind a variety of reverse transcriptase quantitative polymerase chain reaction (RT-qPCR) / PCR inhibitors found in clinical 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 peptides, salts, stabilizers, buffers, sodium azide and proprietary nanoparticles to achieve these tasks. Reagent 123 allows for nucleic acid extraction-free processing of diverse sample types without centrifugations or other sample manipulations, which may introduce errors, contaminants, and/or skew the representation of RNA fragments.

WARNINGS & PRECAUTIONS

For Research Use Only.

- Observe approved biohazard precautions and aseptic techniques to prevent contamination of the product. To be used only by adequately trained and qualified personnel.
- Directions should be read and followed carefully.
- Do not re-pack.
- Do not ingest.

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 or 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 123 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 123

Materials Required But Not Provided: Heating device (heating block or thermal cycler), thin walled tube (0.2 ~ 0.6 mL) or 96-well PCR plate, 15 or 50 mL conical, plate sealer, pipette tips, and test sample

Test Procedure: Proper specimen collection, transport, and storage is critical for successful nucleic acid extraction-free processing.

Compatible transport mediums:

- Phosphate buffered saline (PBS)
- BD™ Universal Viral Transport System (UVT)
- 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™
- 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 123 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. Saliva samples should be processed with PCRopsis™ Lysis Beads when working with bacteria, yeast / fungi, and spores

NOTE: **Urine samples** processed with Reagent 123 require the use of PCRopsis™ Support. Urine (10~25 mL) should be centrifuged at >1,400xg for 10 minutes to pellet cells and the supernatant removed, leaving <250 µL of residual urine to resuspend the pellet. This enriched cell sample is then processed with PCRopsis™ Lysis Beads.

NOTE: PCRopsis™ Lysis Beads are recommended when working with difficult to lyse bacteria, yeast / fungi, and spores. Debris-free, liquid samples (~200 µL) are mixed with 0.25g of PCRopsis™ Lysis Beads in a U-bottom 2 mL tube, and vortexed on high for 5~10 minutes and then used as indicated below.

1. Thoroughly mix PCRopsis™ Reagent 123 to ensure homogeneity, but avoid creating bubbles unnecessarily
 1. Reagent 123 has a hazy, white color when homogenized and normal settlement occurs if not regularly mixed
2. Mix 1 volume of Reagent 123 (20 µL) with 1 volume of sample (20 µL) in a thin walled tube (0.2 ~ 0.6 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.**
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: 10~15 minutes

3. Bacteria / Fungi: 15~20 minutes
4. **NOTE:** heating for a longer period of time does not negatively affect results and may improve your gene amplification
2. Make sure the heating device has reached the desired temperature before applying sample.
3. You may need to increase the heating time if increasing the volume of sample and reagent past 100 µl of each
4. Sample heating can be performed using a controlled heating block or thermal cycler; however a device lid is highly recommended to minimize popping of tube caps or unpeeling of the plate sealer
5. Mix heated sample and use lysed / stabilized sample in your desired PCR procedure
 1. Lysed / stabilized sample should represent ~25% (15% ~ 30%) of your final PCR mixture (i.e., 3~6 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

Quality Control: All lots of PCRopsis™ Reagent 123 are tested for microbial contamination and the ability to perform nucleic acid extraction-free sample processing from liquid samples.

AVAILABILITY – NOT FOR RESALE

Cat. #	Description
1230001	PCRopsis™ Reagent 123, 1 mL
1230025	PCRopsis™ Reagent 123, 25 mL
1230100	PCRopsis™ Reagent 123, 100 mL
1231000	PCRopsis™ Reagent 123, 1000 mL

MANUFACTURER

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

Glossary of Symbols Used

	Research use only		Keep away from direct sunlight
	Manufacturer's catalog number		Number of tests
	Lot number		Consult instructions for use
	Expiration date (year/month)		Sterile through aseptic techniques
	Storage temperature		Manufacturer