



## PCRopsis™ Stool Kit (beta version) ( NOT FOR RESALE )

### INTENDED USE (research use only)

PCRopsis™ Stool Kit is intended for nucleic acid extraction-free processing of stool samples.

### PRINCIPLES OF THE PROCEDURE

PCRopsis™ Stool Kit is engineered to simultaneously bind a variety of reverse transcriptase quantitative polymerase chain reaction (RT-qPCR) / PCR inhibitors found in stool 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 molecules to achieve these tasks. The PCRopsis™ Stool Kit allows for nucleic acid extraction-free processing with minimal sample manipulation.

### 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 the reagents or beads.

**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 10–25°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™ Stool Kit 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™ Clean Buffer A, PCRopsis™ Reagent Clean, PCRopsis™ Activator, PCRopsis™ Lysis Beads

**Materials Required But Not Provided:** Heating device (heating block or thermal cycler), centrifuge, vortexer, thin walled tube (0.2 ~ 0.6 mL) or 96-well PCR plate, 2 mL round-bottom tubes, plate sealer, pipette tips and test sample

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

**Compatible stool transport mediums:**

- Cary Blair Medium
- Phosphate buffered saline (PBS)

**Transport Mediums Not Recommended:**

- Mediums containing guanidinium thiocyanate, alcohols, or other enzyme inhibitors

**Stool Samples (unmodified or in compatible transport medium):**

- solid stool
  - liquid stool
  - samples in Cary Blair medium should be homogenized through vortexing and heating (if necessary)
1. Mix ~50  $\mu$ L or ~50 mg of stool sample with 450  $\mu$ L of PCRopsis™ Clean Buffer A in a 2 mL round-bottom tube containing ~0.25 g PCRopsis™ Lysis Beads
    1. One full PCRopsis™ Lysis Bead Scoop holds ~0.3 grams of beads
    2. The stool sample can be mixed with the lysis beads in alternative tube types and sizes as long as the lysis beads move freely when vortexed in the following step; non-tapered tubes tend to work best
  2. Vortex mixture for 5 minutes on high at room temperature to release and lyse microorganisms
  3. Centrifuge vortexed sample for 5 minute at ~3000 RPM to pellet dense material, resulting in two phases with a clarified lysate on the top phase
  4. Immediately transfer the top, clarified lysate phase to a fresh 1.5 mL tube
  5. Mix 995  $\mu$ L PCRopsis™ Reagent Clean with 5  $\mu$ L PCRopsis™ Activator
    1. Referred to as: Activated Reagent Clean
    2. This mixture is stable for ~4 hours at room temperature and ~24 hours at 4°C
  6. Mix 1 volume of Activated Reagent Clean (20  $\mu$ L) with 1 volume of clarified lysate (20  $\mu$ L) in a thin walled tube (0.2 ~ 0.6 mL) or 96-well PCR plate
    1. **For optimal results, the Activated Reagent Clean needs to be added first to the tube before the sample is added.**
    2. Thoroughly mix PCRopsis™ Reagent Clean to ensure homogeneity before the addition of Activator, but avoid creating bubbles unnecessarily
      1. Reagent Clean has a hazy, white color when homogenized and normal settlement occurs if not regularly mixed
  7. Pipette up & down to ensure complete mixing and then cap tube or apply plate sealer to plate to prevent evaporation
  8. Heat diluted sample for 15~20 minutes at 95°C and let cool at room temperature for 10 seconds before continuing
    1. NOTE: heating for a longer period of time does not negatively affect results and may improve your amplification limit
    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  $\mu$ l 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
9. 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  $\mu$ L sample into a total volume of 20  $\mu$ L)
  2. You might observe increasing PCR inhibition when your PCR mixture consist of >30% processed sample

**Quality Control:** All lots of PCRopsis™ Clean Buffer A, PCRopsis™ Reagent Clean, PCRopsis™ Activator, and PCRopsis™ Lysis Beads are tested for microbial contamination and the ability to perform nucleic acid extraction-free sample processing.

### AVAILABILITY – NOT FOR RESALE

Cat. #	Description
78600100	PCRopsis™ Stool Kit (100 tests)
78601000	PCRopsis™ Stool Kit (1,000 tests)
78610000	PCRopsis™ Stool Kit (10,000 tests)

### MANUFACTURER

Entopsis, Inc., 7600 NW 69th Ave, Medley, FL 33166, USA [info@entopsis.com](mailto:info@entopsis.com)

### Glossary of Symbols Used

<div style="border: 1px solid black; padding: 2px; display: inline-block; margin-right: 5px;"><b>RUO</b></div> Research use only		Keep away from direct sunlight
<div style="border: 1px solid black; padding: 2px; display: inline-block; margin-right: 5px;"><b>REF</b></div> Manufacturer's catalog number		Number of tests
<div style="border: 1px solid black; padding: 2px; display: inline-block; margin-right: 5px;"><b>LOT</b></div> Lot number		Consult instructions for use
 Expiration date (year/month)	<div style="border: 1px solid black; padding: 2px; display: inline-block; margin-right: 5px;"><b>STERILE A</b></div>	Sterile through aseptic techniques
 Storage temperature		Manufacturer