PCR*opsis*™ Stool Kit

(NOT FOR RESALE) v.20230220

INTENDED USE (Research use only. Not for use in diagnostic procedures.)
PCR*opsis*™ Stool Kit is intended for nucleic acid extraction from stool samples.

PRINCIPLES OF THE PROCEDURE
PCR*opsis*™ Stool Kit is engineered to simultaneously bind a variety of inhibitors found in stool, lyse microorganisms, and stabilize RNA and DNA in a manner that's compatible with downstream applications. The product consist of a mixture of peptides, salts, stabilizers, buffers, and sodium azide to achieve these tasks. The Stool Kit allows for nucleic acid extraction with minimal sample manipulation.

WARNINGS & PRECAUTIONS
For Research Use Only.
- Observe approved biohazard precautions and aseptic techniques to prevent contamination of the product.
- 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 printed on the label.

Product Deterioration: PCR*opsis*™ 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: PCR*opsis*™ Clean Buffer A, PCR*opsis*™ Reagent Clean, PCR*opsis*™ Activator, PCR*opsis*™ 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

Stool Samples (unmodified or in transport medium):
- solid stool
- liquid stool
- samples in Cary Blair medium should be homogenized through vortexing and heating (if necessary)

1. Mix ~50 µL or ~50 mg of stool sample with 450 µL of PCR*opsis*™ Clean Buffer A in a 2 mL round-bottom tube containing ~0.25 g PCR*opsis*™ Lysis Beads
    1. One full PCR*opsis*™ 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 500 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 µL PCR*opsis*™ Reagent Clean with 5 µL PCR*opsis*™ Activator
    1. Referred to as: Activated Reagent Clean
    2. This mixture is stable for ~24 hours at room temperature and ~48 hours at 4˚C

6. Mix 1 volume of Activated Reagent Clean (20 µL) with 1 volume of clarified lysate (20 µL) 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 PCR*opsis*™ 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 may improve the nucleic acid extraction efficiency
    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

9. Mix heated sample and use lysed / stabilized sample in your desired downstream applications

AVAILABILITY – NOT FOR RESALE
One extraction refers to processing one sample

Cat. #	Description		
78600100	PCR*opsis*™ Stool Kit	100	extractions
78601000	PCR*opsis*™ Stool Kit	1,000	extractions
78610000	PCR*opsis*™ Stool Kit	10,000	extractions

MANUFACTURER
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Glossary of Symbols Used

RUO	Research use only		Storage temperature
REF	Manufacturer's catalog number	STERILE A	Sterile through aseptic techniques
LOT	Lot number		Manufacturer
	Expiration date (year/month)		