PCR*opsis*™ Stool Kit (beta version)

(NOT FOR RESALE)

INTENDED USE (Research Use Only)

PCR*opsis*™ Stool Kit is intended as an extraction-less procedure performed on properly collected and transported stool specimens in compatible transport mediums before nucleic acid amplification of RNA or DNA.

PRINCIPLES OF THE PROCEDURE

PCR*opsis*™ 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 PCR*opsis*™ Stool Kit allows for extraction-free amplification of RNA / DNA without performing nucleic acid extraction / isolation.

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.
- Pathogenic microorganisms, including hepatitis viruses and Human Immunodeficiency Virus, may be present in clinical specimens. "Standard Precautions"[1-4] and institutional guidelines should be followed in handling all potentially bio-hazardous materials.
- 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 reagents or beads.
- 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 10–25˚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: 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

Test Procedure: Proper specimen collection, transport, and storage is critical for successful nucleic acid amplification. For specific guidance regarding specimen collection procedures, consult published reference manuals.[5-11] Clinical specimens should be collected as soon as possible after the clinical onset of disease. Highest viral titers are present during the acute illness.

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

1. Mix ~50 µL or ~50 mg of stool sample with 500 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 1 minute at 5000 RPM to pellet dense material, resulting in two phases with a clarified lysate on the top phase
4. 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 ~4 hours at room temperature and ~24 hours at 4˚C
5. 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

6. Pipette up & down to ensure complete mixing and then cap tube or apply plate sealer to plate to prevent evaporation
7. Heat diluted sample for 10 minutes at 95˚C and let cool at room temperature for 10~20 seconds before continuing
    1. NOTE: heating for a longer period of time does not negatively affect results and may improve your detection 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 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
8. Mix heated sample and use lysed / stabilized sample in your desired PCR procedure
    1. Lysed / stabilized sample should represent 15% ~ 30% of your final PCR mixture (i.e., 3~6 µL sample into a total volume of 20 µL) depending on the polymerase used
    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 ~ 50˚C for **30 minutes** (extended step improves sensitivity)
        b. 95˚C for 2 minutes
    2. PCR amplification (~40 cycles):
        a. 95˚C for 30 seconds
        b. 55˚C for 30 seconds
        c. 72˚C for 30 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

Quality Control: All lots of PCR*opsis*™ Clean Buffer A, PCR*opsis*™ Reagent Clean, PCR*opsis*™ Activator, and PCR*opsis*™ Lysis Beads are tested for microbial contamination and the ability to amplify RNA / DNA without nucleic acid extraction. If aberrant quality control results are noted, patient results should not be reported.

RESULTS

Results obtained will partially depend on proper and adequate specimen collection, transport, and processing in the laboratory. The use of PCR*opsis*™ Stool Kit with incompatible transport mediums or mediums with noticeable microbial growth (i.e., contamination) may result in unreliable results.

LIMITATIONS OF THE PROCEDURE

- The performance characteristics of the PCR*opsis*™ Stool Kit were validated using endogenous bacteria and yeast found in stool through qPCR in compatible transport mediums. The use of alternative microorganisms, transport mediums, gene targets and / or detection methods may affect the performance of the product.
- RT-qPCR / PCR cycle thresholds (Ct) should be set higher than when extracted RNA / DNA is utilized for amplification (e.g., 40~45 cycles).
- Repeated freezing and thawing of test specimens may reduce the detection of desired gene targets.
- Follow recommended guidelines for specimen collection, transport and storage as this may affect the ability to amplify gene targets.

PERFORMANCE CHARACTERISTICS

The performance of the PCR*opsis*™ Stool Kit will be compared to alternative approaches in the future.

AVAILABILITY – NOT FOR RESALE

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

MANUFACTURER

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REFERENCES

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Glossary of Symbols Used

IVD	In vitro diagnostic use		Keep away from direct sunlight
REF	Manufacturer's catalog number	Σ	Number of tests
LOT	Lot number		Consult instructions for use
	Expiration date (year/month)	STERILE A	Sterile through aseptic techniques
	Storage temperature		Manufacturer