SUGGESTED PROTOCOL:
Extraction-free Processing of Stool

v. 20230220

Sample:

- Solid stool
- Liquid stool: homogenized through vortexing and heating (if necessary)
    - Compatible transport mediums:
        - Cary Blair Medium
        - Phosphate buffered saline (PBS)

Materials:

- Entopsis Inc.:
    - PCR*opsis*™ Stool Kit (see product IFU)
- 2 mL round-bottom tube
- Test sample

Methods:

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 5~10 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 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 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
    1. For PCR amplification of extracted RNA / DNA: 3-step PCR typically outperforms 2-step PCR