PCR*opsis*™ Lysis Beads

(NOT FOR RESALE)

v.20231010

INTENDED USE (Research use only. Not for use in diagnostic procedures.)
PCR*opsis*™ Lysis Beads are intended to facilitate lysis of difficult to lyse microorganisms.

PRINCIPLES OF THE PROCEDURE
PCRopsis™ Lysis Beads are solid beads coated with a mixture of molecules that facilitate lysis of difficult to lyse microorganisms and ensure compatibility with PCR*opsis*™ reagents.

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.

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 ~25˚C until used. Do not overheat or keep open in humid environments. Do not 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*™ Lysis Beads should not be used if (1) there is evidence of damage or contamination to the product, (2) there is evidence of moisture in the original container, (3) the color of the beads has changed from white-clear prior to use, (4) the expiration date has passed, or (5) there are other signs of deterioration.

PROCEDURES

Materials Provided: PCR*opsis*™ Lysis Beads

Materials Required But Not Provided: PCR*opsis*™ Reagent RVD with RVD Enhancer (see product IFU), PCR*opsis*™ Support (see product IFU), PCR*opsis*™ Lysis Bead Scoop, nuclease-free water, 1.5 mL tube or 2 mL deep well plate, thermal cycler, controlled heat block, thin walled tube (0.2 ~ 0.6 mL) or 96-well PCR plate, plate sealer, pipette tips and test sample

Specimen: liquid sample containing microorganisms (e.g., dairy, waste water, centrifuged urine, oral rinse, beverages, swab in compatible medium, toenails in compatible medium, etc.)

Procedure Outlines Based on Sample Types:

In all cases, samples should be free of debris. Remove debris from samples by letting the debris settle for ~10 minutes with gravity, centrifuging the sample at a low speed, or filtering the sample and only using the debris-free sample for processing.

The Lysis Beads may clump and this is normal. Clumps are easily broken apart by tapping, shaking, or vortexing the container, or by mechanical force with a sterile spatula or similar device.

For urine-based samples:

PCR*opsis*™ Reagent Prep:

1. Thoroughly mix 2 µL of PCR*opsis*™ Support with 1 mL PCR*opsis*™ Reagent RVD with RVD Enhancer (called **RVD-Support solution** from here on)
    1. This mixture is stable for at least 24 hours at room temperature

After Reagent Prep:

1. Centrifuge 1.5~25 mL of urine at >1,400xg for 10 minutes in a 15 mL or 50 mL conical
    1. Higher volumes of centrifuged urine is expected to result in higher sensitivity

2. Remove supernatant and leave <250 µL of residual urine

3. Resuspend cell pellet using residual urine

4. Add roughly 0.25 grams of Lysis Beads to a 1.5 ~ 2 mL vial or well in a deep, 96-well plate
    1. One full PCR*opsis*™ Lysis Bead Scoop holds ~0.3 grams of beads

5. Add 200 µL of sample to the vial or well containing Lysis Beads
    1. A 2 mL, round-bottom tubes works best

6. Cap tube or place a plate sealer on the deep well plate

7. Vortex on high for ~5 minutes to lyse microorganisms

8. Mix 20 µL PCR*opsis*™ RVD-Support solution with 20 µL of the sample pre-processed with PCR*opsis*™ Lysis Beads in a thin-walled tube or plate (0.2 ~ 0.6 mL)
    1. **For optimal results, the reagent needs to be added first to the tube/well before the sample is added.**
    2. Ratio of sample to PCR*opsis*™ reagent will remain 1:1, but volume can be increased if needed (example: 30 µl : 30 µl and so forth)

9. Pipette up & down to ensure complete mixing and then cap tube or apply plate sealer to plate to prevent evaporation

10. Heat diluted sample at 95˚C for 15~20 minutes in a thermal cycler or controlled heat block with a lid, and let cool at room temperature for ~10 seconds before continuing
    1. 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

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

For toenail samples:

PCR*opsis*™ Reagent Prep:

None needed. Please use PCR*opsis*™ Reagent RVD with RVD Enhancer directly.

After Reagent Prep:

1. Cut toe nail samples into small pieces measuring less than 0.5 cm in length to maximized exposed surface area

2. Add roughly 0.25 grams of Lysis Beads to a 1.5 ~ 2 mL vial or well in a deep, 96-well plate
    1. One full PCR*opsis*™ Lysis Bead Scoop holds ~0.3 grams of beads

3. Place nail pieces into a vial or well with Lysis Beads
    1. A 2 mL, round-bottom tubes works best

4. Add ~200 µL of nuclease-free water to the vial or well with nail pieces and Lysis Beads

5. Cap tube or place a plate sealer on the deep well plate

6. Vortex on high for ~5 minutes to lyse microorganisms

7. Mix 20 µL PCR*opsis*™ Reagent RVD with RVD Enhancer with 20 µL of the sample pre-processed with PCR*opsis*™ Lysis Beads in a thin-walled tube or plate (0.2 ~ 0.6 mL)
    1. **For optimal results, the reagent needs to be added first to the tube/well before the sample is added.**
    2. Ratio of sample to PCR*opsis*™ reagent will remain 1:1, but volume can be increased if needed (example: 30 µl : 30 µl and so forth)

8. Pipette up & down to ensure complete mixing and then cap tube or apply plate sealer to plate to prevent evaporation

9. Heat diluted sample at 95˚C for 15~20 minutes in a thermal cycler or controlled heat block with a lid, and let cool at room temperature for ~10 seconds before continuing
    1. 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

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

<u>For select liquid samples (beverages, water, swab samples in transport mediums, oral rinse, saliva samples, etc.)</u>:

PCR*opsis*™ Reagent Prep:

None needed. Please use PCR*opsis*™ Reagent RVD with RVD Enhancer directly.

After Reagent Prep:

<u>OPTIONAL</u>:

1. Centrifuge 1.5~25 mL of sample at >1,400xg for 10 minutes in a 15 mL or 50 mL conical to concentrate cells
    1. Higher volumes of centrifuged sample is expected to result in higher concentrations of nucleic acids for downstream applications
2. Remove supernatant and leave <250 µL of residual sample

1. Add roughly 0.25 grams of Lysis Beads to a 1.5 ~ 2 mL vial or well in a deep, 96-well plate
    1. One full PCR*opsis*™ Lysis Bead Scoop holds ~0.3 grams of beads

2. Add 200 µL of sample to the vial or well containing Lysis Beads
    1. A 2 mL, round-bottom tubes works best

3. Cap tube or place a plate sealer on the deep well plate

4. Vortex on high for ~5 minutes to lyse microorganisms

5. Mix 20 µL PCR*opsis*™ Reagent RVD with RVD Enhancer with 20 µL of the sample pre-processed with PCR*opsis*™ Lysis Beads in a thin-walled tube or plate (0.2 ~ 0.6 mL)
    1. **For optimal results, the reagent needs to be added first to the tube/well before the sample is added.**

   2. Ratio of sample to PCR*opsis*™ reagent will remain 1:1, but volume can be increased if needed (example: 30 µl : 30 µl and so forth)

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 at 95˚C for 15~20 minutes in a thermal cycler or controlled heat block with a lid, and let cool at room temperature for ~10 seconds before continuing
   1. 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

8. Mix heated sample and use lysed / stabilized sample in your desired downstream application

AVAILABILITY – NOT FOR RESALE
One sample refers to 0.25 g of lysis beads

Cat. #	Description		
5970010	PCR*opsis*™ Lysis Beads, 10 grams (bulk)	40	samples
5970025	PCR*opsis*™ Lysis Beads, 25 grams (bulk)	100	samples
5970500	PCR*opsis*™ Lysis Beads, 500 grams (bulk)	2000	samples
5971000	PCR*opsis*™ Lysis Beads, 1 kilogram (bulk)	4000	samples
5972500	PCR*opsis*™ Lysis Beads, 2.5 kilograms (bulk)	10000	samples

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

PCRopsis
by Entopsis

Glossary of Symbols Used

Symbol	Meaning	Symbol	Meaning
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)		