# PCR*opsis*™ Lysis Beads

**( NOT FOR RESALE )**

**INTENDED USE (in vitro diagnostic use)**

PCR*opsis*™ Lysis Beads are intended to facilitate lysis of difficult to lyse microorganisms.

**PRINCIPLES OF THE PROCEDURE**

PCR*opsis*™ Lysis Beads are solid beads (~0.5 mm diameter) coated with a proprietary coating that facilitate lysis of difficult to lyse microorganisms and ensure compatibility with Next Generation Direct PCR reagents. This coating offers superior performance compared to uncoated beads when used with PCR*opsis*™ direct PCR reagents.

**WARNINGS & PRECAUTIONS**

For in vitro Diagnostic Use.

- Observe approved biohazard precautions and aseptic techniques to prevent contamination of the product. To be used only by adequately trained and qualified personnel.
- 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 reagent.
- 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 4–25˚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 clearly 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), 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

**Test Procedure**: Proper specimen collection, transport and storage is critical for successful nucleic acid amplification.

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

## Procedure Outlines Based on Sample Types:

## 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
    2. If poor results are initially observed, then add 10 ~ 20 µL of PCR*opsis*™ Support per 1 mL PCR*opsis*™ Reagent RVD with RVD Enhancer

**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 slightly longer period (extra 2~5 minutes) of time does not negatively affect results
    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 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

## 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 slightly longer period (extra 2~5 minutes) of time does not negatively affect results
    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 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

## For swab samples in transport mediums or oral rinse / saliva samples:

**PCR*opsis*™ Reagent Prep:**

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

**After Reagent Prep:**

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
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    1. Heating for a slightly longer period (extra 2~5 minutes) of time does not negatively affect results
    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

**Quality Control**: All lots of PCR*opsis*™ Lysis Beads are tested for microbial contamination and the ability to improve the amplification of fungal / yeast targets. If aberrant quality control results are noted, results should not be reported.

## RESULTS

Results obtained will partially depend on proper and adequate specimen collection, transport, and processing in the laboratory.

## LIMITATIONS OF THE PROCEDURE

- Performance characteristics of PCR*opsis*™ Lysis Beads, in combination with PCR*opsis*™ Reagent RVD with RVD Enhancer containing PCR*opsis*™ Support, were validated using human urine spiked with Candida albicans. Fungal DNA was amplified through PCR. The use of alternative samples, reagents and / or detection methods may affect the performance of the product.
- Repeated freezing and thawing of test specimens may reduce the detection of desired gene targets.
- The RVD-Support solution + sample mixture must be used within 4 hours for downstream PCR applications after the 10-minute incubation at 95˚C to ensure optimal results.
- PCR*opsis*™ Lysis Beads should not be washed before use since it will result in diminished functionality.
- Follow recommended guidelines for specimen collection, transport, and storage as this may affect the ability to amplify gene targets.

## PERFORMANCE CHARACTERISTICS

The performance of PCR*opsis*™ Lysis Beads in combination with PCR*opsis*™ Reagent RVD with RVD Enhancer containing PCR*opsis*™ Support was confirmed using human urine spiked with Candida albicans as the test sample. The test sample was processed as recommended and fungal-specific 16S rRNA gene was amplified through PCR.

## AVAILABILITY – NOT FOR RESALE

| Cat. # | Description |
|---|---|
| 5970025 | PCR*opsis*™ Lysis Beads, 25 grams (bulk) |
| 5970500 | PCR*opsis*™ Lysis Beads, 500 grams (bulk) |
| 5971000 | PCR*opsis*™ Lysis Beads, 1 kilogram (bulk) |
| 5972500 | PCR*opsis*™ Lysis Beads, 2.5 kilograms (bulk) |

## MANUFACTURER

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

PCRopsis by Entopsis

## Glossary of Symbols Used

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