PCR*opsis*™ Elution Buffer

(NOT FOR RESALE)

INTENDED USE (in vitro diagnostic use)

PCR*opsis*™ Elution Buffer is intended for the elution of test material from substrates.

PRINCIPLES OF THE PROCEDURE

PCR*opsis*™ Elution Buffer is engineered to elute test material from substrates in a manner that's compatible with PCR*opsis*™ Next Generation Direct PCR™ reagents. This buffer uses a mixture of salts, peptides, and proteins to extract relevant test material (e.g., RNA / DNA) from substrates, like filter paper.

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.
- 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 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. Do not incubate or 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*™ Elution Buffer 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, (4) the expiration date has passed, or (5) there are other signs of deterioration.

PROCEDURES

Materials Provided: PCR*opsis*™ Elution Buffer

Materials Required But Not Provided: PCR*opsis*™ Reagent RVD with RVD Enhancer, hole puncher, heating device (heating block or thermal cycler), 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. 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.

Specimen: blood dried onto Whatman Filter paper

1. Create hole punches of your test sample using a standard, sterile hole puncher
2. Place 1 hole punched filter paper sample into a 1.5 mL tube
3. Add ~100 µL of PCR*opsis*™ Elution Buffer to the tube
4. Vortex thoroughly for 1~3 minutes to elute material off the filter paper
5. Mix 20 µL PCR*opsis*™ Reagent RVD with RVD Enhancer with 20 µL of eluted sample in a thin-walled tube (0.2 ~ 0.6 mL)
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 10 minutes and let cool at room temperature for ~10 seconds before continuing
    1. Heating for a slightly longer period (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. Sample heating can be performed using a controlled heating block or thermal cycler
8. Use the top, clear phase of the heated 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*™ Elution Buffer are tested for microbial contamination and the ability to elute nucleic acids from Whatman filter paper. 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.

LIMITATIONS OF THE PROCEDURE

- Performance characteristics of PCR*opsis*™ Elution Buffer were validated using human blood dried onto Whatman Filter paper in combination with PCR*opsis*™ Reagent RVD with RVD Enhancer where the eluted 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.
- 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*™ Elution Buffer in combination with PCR*opsis*™ Reagent RVD with RVD Enhancer was confirmed using human blood dried onto Whatman Filter paper as the test sample. The test sample was processed as recommended and a segment of the GAPDH gene was amplified through PCR.

AVAILABILITY – NOT FOR RESALE

Cat. #	Description
358001	PCR*opsis*™ Elution Buffer, 1 mL (for validation purposes only)
358025	PCR*opsis*™ Elution Buffer, 25 mL
358100	PCR*opsis*™ Elution Buffer, 100 mL
358500	PCR*opsis*™ Elution Buffer, 500 mL
3581000	PCR*opsis*™ Elution Buffer, 1000 mL

MANUFACTURER

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

REFERENCES

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Glossary of Symbols Used