



ZooOpsis™ Reagent Swab (beta)

(Not for Resale)

INTENDED USE (research use only)

ZooOpsis™ Reagent Swab is intended for extraction-free processing of specimens on swabs before PCR, without the need for transport mediums. Not intended for human clinical samples.

PRINCIPLES OF THE PROCEDURE

ZooOpsis™ Reagent Swab is engineered to simultaneously elute material from swabs, bind a variety of reverse transcriptase quantitative polymerase chain reaction (RT-qPCR) / PCR inhibitors found in clinical samples, lyse specimens 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, and sodium azide to achieve this task. Reagent Swab allows for extraction-free amplification of nucleic acids without performing extractions, centrifugations or other sample manipulations, which may introduce errors, contaminants and/or skew the representation of RNA fragments.

WARNINGS & PRECAUTIONS

For Research Use Only (RUO).

- 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 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 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: ZooOpsis™ Reagent Swab 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: ZooOpsis™ Reagent Swab

Materials Required But Not Provided: Thermal cycler, heating device (heating block or thermal cycler), tube (0.2 ~ 0.6 mL) or 96-well PCR plate, plate sealer, pipette tips, PCR kit with primers / probe, and test sample

Test Procedure: Collect test specimen using a flocked nylon Swab or similar. 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 Specimens should be collected as soon as possible after the onset of disease. Highest viral titers are present during the acute illness.

Recommended swabs: synthetic swabs (nylon, rayon, dacron, polyester) with aluminum or plastic shafts

Material to be tested: specimen-containing swab properly transported in a sterile tube

1. Thoroughly mix Reagent Swab to ensure homogeneity, but avoid creating bubbles unnecessarily
 1. Reagent Swab has a hazy, white color when homogenized and normal settlement occurs if not regularly mixed
2. Elute material from Swab:
 1. Add ~100 μ L of Reagent Swab to a tube with the specimen-containing swab
 2. Make sure the swab is at least partially submerged into Reagent Swab
 3. Vortex for ~30 seconds, 3 times, to elute sample
3. Specimen lysis & nucleic acid stabilization:
 1. Transfer ~50 μ L of eluted sample into a thin-walled PCR tube / plate and then cap tube or apply plate sealer to plate to prevent evaporation
 2. Heat at 95°C
 1. Mammalian: 5 minutes
 2. Viruses: 10~15 minutes
 3. Bacteria: 15 ~ 20 minutes
 4. NOTE: heating for a longer period of time does not negatively affect results
 3. Let cool at room temperature for ~10 seconds before continuing
4. Pipette up & down to ensure complete mixing
5. Use lysed / stabilized sample in your desired RT-qPCR / qPCR procedure
 1. Lysed / stabilized sample should represent 15%~30% of your final RT-qPCR mixture (i.e., 3~6 μ L sample into a total volume of 20 μ L)
 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 for 15 minutes (extend to 30 minutes if suboptimal results observed)
 - b. 95°C for 2 minutes
2. PCR amplification (~40 cycles):
 - a. 95°C for 5 seconds
 - b. 55°C for 15 seconds
 - c. 72°C for 15 seconds
3. Hold: 4°C

NOTE:

- When amplifying RNA, 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 ZooOpsis™ Reagent Swab are tested for microbial contamination and the ability to amplify viral RNA without extraction. 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. ZooOpsis™ Reagent Swab may result in unreliable results when used beyond the intended use.

LIMITATIONS OF THE PROCEDURE

- Performance characteristics of ZooOpsis™ Reagent Swab were validated using SARS-CoV-2, *S. aureus* and *P. aeruginosa* dried on flocked nylon swabs. The use of alternative microorganisms, swabs, gene targets and / or detection methods may affect the performance of the product.
- Improper transport and storage of test swabs 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 ZooOpsis™ Reagent Swab was compared to traditional RNA extraction methods (e.g., Qiagen's QIAamp Viral RNA Kit) from the same samples. These studies used SARS-CoV-2 absorbed and dried onto flocked nylon swabs, spiked samples processed using both methods and RT-qPCR was performed using IDT qPCR probe assay and Promega GoTaq® Probe 1-Step RT-qPCR System. Observed Ct values between both methods are within 5 Ct of each other.



AVAILABILITY – NOT FOR RESALE

Cat. #	Description
7920001	ZooOpsis™ Reagent Swab, 1 mL
7920025	ZooOpsis™ Reagent Swab, 25 mL
7920100	ZooOpsis™ Reagent Swab, 100 mL
7921000	ZooOpsis™ Reagent Swab, 1000 mL











MANUFACTURER

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

REFERENCES

1. Clinical and Laboratory Standards Institute. 2005. Approved Guideline M29-A3. Protection of laboratory workers from occupationally acquired infections, 3rd ed. CLSI, Wayne, Pa.
2. Garner, J.S. 1996. Hospital Infection Control Practices Advisory Committee, U.S. Department of Health and Human Services, Centers for Disease Control and Prevention. Guideline for isolation precautions in hospitals. *Infect. Control Hospital Epidemiol.* 17: 53-80.
3. U.S. Department of Health and Human Services. 1999. Biosafety in microbiological and biomedical laboratories, HHS Publication (CDC), 4th ed. U.S. Government Printing Office, Washington, D.C.
4. Directive 2000/54/EC of the European Parliament and of the Council of 18 September 2000 on the protection of workers from risks related to exposure to biological agents at work (seventh individual directive within the meaning of Article 16(1) of Directive 89/391/EEC). *Official Journal L262*, 17/10/2000, p. 0021-0045.
5. Murray, P.R., E.J. Baron, J.H. Jorgensen, M.A. Pfaller, and R.H. Tenover. 2003. *Manual of clinical microbiology*. 8th ed. ASM, Washington, D.C.
6. Gleaves, C.A., R.L. Hodinka, S.L.G. Johnston, and E.M. Swierkosz. 1994. *Cumitech 15A. Laboratory diagnosis of viral infections*. ASM, Washington, DC.
7. Forbes, B.A., D.F. Sahm, and A.S. Weissfeld. 2002. *Bailey and Scott's diagnostic microbiology*. 11th ed. Mosby, St. Louis, MO.
8. Wardford, A., M. Chernesky, and E. M. Peterson. 1999. *Cumitech 19A, Laboratory diagnosis of Chlamydia trachomatis infections*. ASM, Washington, DC.
9. Miller, J. M. 1999. *A guide to specimen management in clinical microbiology*, 2nd ed. ASM, Washington, DC.
10. Isenberg, H. D., 2004. *Clinical microbiology procedures handbook*, 2nd ed. ASM, Washington, DC.
11. Isenberg, H.D., 1998. *Essential procedures for clinical microbiology*. Chapter 14.12, Page 787. Packaging and shipping infectious substances.

Glossary of Symbols Used

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