



Genfind[®] DNA Extraction Kit

REF 95-449

Intended Use: Kit for DNA Extraction



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REAGENTS PROVIDED AND STORAGE REQUIREMENTS

Note: For information on any hazard and precautionary statements that may be associated with reagents, refer to the Safety Data Sheet Library at www.hologic.com/sds.

Table 1: Genfind DNA Extraction Kit (REF 95-449) Contents and Storage Requirements

Reagent	Label Abbreviation	Component Description	Storage Requirement
Genfind Proteinase K	PK	Lyophilized Enzyme (1 mL vials) Ultrapure	-30° to -15°C Store Frozen
Genfind Lysis Buffer	LB	Cell Lysis Solution 0.45 µm filtered	15° to 30°C Store at room temperature
Genfind Binding Buffer	BB	Magnetic Bead Solution 0.45 µm filtered	2° to 8°C Refrigerate – Do Not Freeze
Genfind Wash Buffer	WB	DNA Wash Buffer (Label marked with blue stripes) 0.45 µm filtered	15° to 30°C Store at room temperature

WARNINGS AND PRECAUTIONS

- For *in vitro* diagnostic use.
- Multiple storage conditions exist; see Table 1.
- Universal safety precautions should be used when handling any human tissues or fluids. Specimens should be disposed of according to local requirements.
- Follow good laboratory practices. Wear protective disposable gloves, laboratory coats, and eye protection when handling specimens and kit reagents. Wash hands thoroughly after handling specimens and reagents.

5. Do not pool reagents from different lots or from different vials/bottles of the same lot.
6. Do not use reagents after their expiration date.
7. Prior to use, the Proteinase K lyophilized enzyme should be dissolved in nuclease-free water. A volume of 1 mL of water should be added to each vial as needed. When resuspended with water, the 1 mL vial of Proteinase K should be divided into aliquots and again frozen at -30° to -15°C in a non-frost-free freezer. Thaw only as much Proteinase K as needed for each extraction. Repeated freezing and thawing of the enzyme can cause a loss of function.
8. If a white precipitate has formed in the Wash Buffer, prior to use, gently shake or stir at room temperature until the solids dissolve. Do not heat to recombine.
9. Product components (product residuals, packaging) can be considered as laboratory waste. Dispose of unused reagents and waste in accordance with applicable federal, state, and local regulations.

MATERIALS REQUIRED, BUT NOT PROVIDED

Table 2: Materials required, but not provided. See Table 5 for a list of recommended supplies and equipment.

	96-well Plate Method	Tube Method
Consumable Supplies	<ul style="list-style-type: none"> • Pipette tips, filter barrier • 96-well plates • Foil Plate Sealers • ABgene® 96-well 2.2 mL plates • Nuclease-free disposable tubes and screw caps 	<ul style="list-style-type: none"> • Pipette tips, filter barrier • Nuclease-free disposable tubes and screw caps
Reagents	<ul style="list-style-type: none"> • 2M Tris, pH 7.5 • Nuclease-free water • 70% Ethanol (Molecular Biology grade) • Conversion Solution 	<ul style="list-style-type: none"> • 2M Tris, pH 7.5 • Nuclease-free water • 70% Ethanol (Molecular Biology grade)

	96-well Plate Method	Tube Method
Equipment	<ul style="list-style-type: none"> • Pipettes • Vortex • Plate Centrifuge and rotors • SPRI® Plate 96R Super Magnet Plate • Thermomixer R (Eppendorf) • MTP Block (Eppendorf) and 96 well adapter plate • Digital Dry Block Heater 120 (VWR) • Modular Heating Block for Titer Plates (VWR) • Cervista® MTA System for automation users 	<ul style="list-style-type: none"> • Pipettes • Vortex • TubeCentrifuge • SPRI Stand Magnetic 6 Tube • Thermomixer R (Eppendorf) • MTP Block (Eppendorf) and 2.0 mL tube adapter plate

PREPARATION OF REAGENTS

Equilibrate all reagents to room temperature prior to use.

1. Prepare a 10mM Tris solution from a 2M, pH 7.5 Tris stock solution. For processing a 96-well plate of samples, a recommended preparation is shown in Table 3.

Table 3: Preparation of 10 mM Tris

Component	Volume
2M Tris, pH 7.5	100 μ L
Nuclease-free Water	19.9 mL
Total Solution Volume	20 mL

2. Combine the Lysis Buffer and Proteinase K (96 μ g/ μ L) in an appropriate-sized conical tube according to Table 4. Mix by pipetting up and down.

Table 4: Preparation of Lysis Buffer

Component	Volume/ Sample	Number of Samples (x)	Total Volume
Lysis Buffer	400 μ L	x	(400 μ L)(x)(1.2)
Proteinase K	9 μ L	x	(9 μ L)(x)(1.2)
LB/PK solution	409 μ L	x	(409 μ L)(x)(1.2)

INSTRUCTIONS FOR USE FOR CERVISTA MTA SYSTEM

Refer to the Cervista MTA Operator’s Manual (P/N MAN-02378-002) for the instructions for use for the Cervista MTA System.

NOTE: PRIOR TO USE OF THE CERVISTA MTA SYSTEM FOR GENFIND DNA EXTRACTION OF THE SUREPATH LIQUID CYTOLOGY SAMPLE THE SAMPLE MUST BE TREATED ACCORDING TO THE SAMPLE CONVERSION PROCEDURE.

INSTRUCTIONS FOR USE FOR MANUAL PROCEDURE

Sample Conversion Procedure

SurePath Liquid Cytology Samples – 96-well Plate Method

NOTE: THE SUREPATH LIQUID CYTOLOGY SAMPLE USED IN THIS METHOD IS THE RESIDUAL ENRICHED PELLET CERVICAL SAMPLE PROCESSED ACCORDING TO THE PREPSTAIN SLIDE PROCESSOR OPERATOR’S MANUAL – PREPSTAIN PROCESS PROCEDURE.

1. Mix the residual enriched pellet cervical sample well by vortexing or shaking vigorously. Transfer 1.0 mL of each sample to a well of a 96-well 2.2 mL plate.
2. Centrifuge the 96 well 2.2 mL plate at approximately 1100 RCF for 10 minutes.
3. Place the 2.2 mL plate on the SPRI Plate 96R Super Magnet Plate. Remove the supernatant using a multi-channel pipette or a 96-well aspirator and pump (pressure of aspirator should be approximately 100 mm Hg vac). Remove the supernatant leaving 50-100 μ L of residual volume. Take care to only remove supernatant and not cellular material. NOTE: IF USING AN ASPIRATOR, RINSE THE ASPIRATOR WITH FRESH DISTILLED WATER FOLLOWING THIS STEP.

4. Add 0.2 mL Conversion Solution to each sample.
5. Incubate the plate on a Digital Dry Block Heater set to 115°C (+/- 2°C) for 60 minutes.
6. Upon incubation completion, remove plate from the heat block.
7. Transfer the entire contents of each converted sample to a well of a second 2.2 mL plate containing 1.59mL of water. NOTE: USE NEW TIPS FOR EACH SAMPLE LIQUID TRANSFER.

For the Manual 96-well plate method, continue to step 2 of the section “PreservCyt® Liquid Cytology Samples and Converted SurePath Liquid Cytology Samples – 96-well Plate Method”.

For the MTA System, refer to the Cervista MTA Operator’s Manual (Part Number MAN-02378-002) for the instructions for use for the Cervista MTA System.

NOTE: IF DESIRED, PRESERVCYT LIQUID CYTOLOGY SAMPLES MAY BE CO-PROCESSED WITH THE GENFIND DNA EXTRACTION PROCESS IN ANY EMPTY WELLS OF THE 2.2 ML PLATE CONTAINING THE CONVERTED SUREPATH SAMPLES AND WATER.

Genfind DNA Extraction Procedure

PreservCyt Liquid Cytology Samples and Converted SurePath Liquid Cytology Samples – 96-well Plate Method

1. Mix the cervical specimen well by vortexing or shaking vigorously. Transfer 2.0 mL of each specimen to a well of a 96-well 2.2 mL plate.
2. Centrifuge the 96 well 2.2 mL plate at approximately 1100 RCF for 10-15 minutes.
3. Place the 2.2 mL plate on the SPRI Plate 96R Super Magnet Plate. Remove the supernatant using a multi-channel pipette or a 96-well aspirator and pump (pressure of aspirator should be approximately 100 mm Hg vac). Remove approximately 1.9 mL of the supernatant leaving 50-100 μ L of residual volume. Take care to only remove supernatant and not cellular material. NOTE: IF USING AN ASPIRATOR RINSE WITH FRESH DISTILLED WATER FOLLOWING STEPS 3, 8, 11, 12, and 14.
4. Add 400 μ L of the Lysis Buffer/proteinase K mixture to each well containing sample of the 96-well plate. NOTE: USE NEW TIPS FOR EACH SAMPLE WELL IN ALL LIQUID TRANSFER STEPS.
5. Incubate the plate on a thermomixer for 15 minutes at 37°C +/-2°C and 1000 rpm. NOTE: AFTER THIS STEP, TURN THE THERMOMIXER THERMOSTAT OFF. THE THERMOMIXER THERMOSTAT SHOULD REMAIN OFF FOR ALL SUBSEQUENT STEPS.

6. **IMPORTANT:** Mix the Binding Buffer thoroughly by inverting the bottle many times, making sure the beads are fully resuspended. After mixing, add 200 μ L to each well containing sample of the 96-well plate.
7. Place the plate on a thermomixer and mix at 1000 rpm for 2-3 minutes.
8. Place the SPRI Plate 96R Super Magnet Plate on the spacer and place the 2.2 mL plate on the magnet for 4-6 minutes or until beads form a distinct ring and solution is clear. Aspirate the entire supernatant taking care not to disturb the beads. **NOTE: USE OF A SPACER IS NECESSARY FOR ALL SUBSEQUENT ASPIRATION STEPS IF USING THE 96 WELL ASPIRATOR AND PUMP.**
9. Remove the plate from the magnet and spacer and add 400 μ L of Wash Buffer to the plate wells containing beads.
10. Place the plate on a thermomixer and mix at 1000 rpm for 4-6 minutes.
11. Place the SPRI Plate 96R Super Magnet Plate on the spacer and place the 2.2 mL plate on the magnet for 4-6 minutes or until beads form a distinct ring and solution is clear. Aspirate entire supernatant taking care to not disturb the beads. **NOTE: The plate should remain on the magnet and spacer during steps 12-14.**
12. Add 400 μ L of 70% ethanol to the wells containing beads and incubate for 30-60 seconds. The beads should form a distinct ring. Aspirate entire supernatant.
13. Repeat the 70% ethanol wash by adding 400 μ L 70% of ethanol to the wells containing beads and incubate for 30-60 seconds. The beads should form a distinct ring. Aspirate entire supernatant.
14. Allow the beads to air dry for 3-4 minutes. **NOTE: IT IS IMPORTANT TO REMOVE ALL RESIDUAL ETHANOL BEFORE PROCEEDING TO THE NEXT STEP.**
15. Remove the plate from the magnet and add 120 μ L of 10mM Tris to each well containing beads.
16. Place the plate on a thermomixer and alternate mixing at:
 - i. 1000 rpm for 2-3 minutes
 - ii. Let stand for 2-3 minutes.
 - iii. 1000 rpm for 2-3 minutes.
17. Place the plate on a magnet for 10 minutes or until beads form a distinct ring and solution is clear.
18. While the plate is still on the magnet, transfer 110 μ L of the DNA solution to a clean 96-well PCR plate using a multi-channel pipette.
19. If the beads are present visually in the DNA solution, place the 96-well PCR plate on the magnet and allow any particles to settle. While the plate is still

on a magnet, transfer 100 μ L of DNA to a clean 96-well PCR plate. Seal the plate with a foil plate sealer.

20. DNA can be stored at 4–8°C for up to four weeks. For storage longer than four weeks, store the sample DNA in a –20° or –80°C non-frost-free freezer.

PreservCyt Liquid Cytology Samples – Tube Method

NOTE: THE TUBE METHOD HAS NOT BEEN VALIDATED FOR USE WITH THE SUREPATH LIQUID CYTOLOGY SAMPLE.

1. Mix the cervical specimen well by vortexing or shaking vigorously. Transfer 2.0 mL of each specimen into a labeled 2.0 mL screw-cap tube and cap the tube.
2. Centrifuge at approximately 1100 RCF for 10-15 minutes.
3. Remove the supernatant using a pipette. Remove approximately 1.9mL of the supernatant leaving 50-100 μ L of residual volume. Take care to only remove supernatant and not cellular material.
4. Add 400 μ L of the Lysis Buffer/proteinase K mixture to each tube. **NOTE: USE NEW TIPS FOR EACH SAMPLE IN ALL LIQUID TRANSFER STEPS.**
5. Incubate the tubes in a thermomixer for 15 minutes at 37°C +/-2°C and 1000 rpm. **NOTE: AFTER THIS STEP, TURN THE THERMOMIXER THERMOSTAT OFF. THE THERMOMIXER THERMOSTAT SHOULD REMAIN OFF FOR ALL SUBSEQUENT STEPS.**
6. **IMPORTANT:** Mix the Binding Buffer thoroughly by inverting the bottle many times, making sure the beads are fully resuspended. After mixing, add 200 μ L to each tube.
7. Place the tubes in the thermomixer and mix at 1000 rpm for 2-3 minutes.
8. Place sample tubes on the magnet for 4-6 minutes and wait for solution to clear. While the tubes are still on the magnet, remove supernatant with a pipette.
9. Remove the tubes from the magnet and add 400 μ L of Wash Buffer to each tube.
10. Place the tubes in the thermomixer and mix at 1000 rpm for 4-6 minutes.
11. Place sample tubes on the magnet for 4-6 minutes and wait for solution to clear. While the tubes are still on the magnet, remove supernatant with a pipette.
12. Add 400 μ L of 70% ethanol to each sample, place the tubes in the thermomixer and mix at 1000 rpm for 1 minute.
13. Place the sample tubes on the magnet for 4-6 minutes and wait for the solution to clear. While the tubes are still on the magnet, remove

- supernatant with a pipette. Note: Tubes should remain on the magnet during steps 14 and 15.
14. Repeat the 70% ethanol wash by adding 400 μ L of 70% ethanol to the samples and let stand for 30-60 seconds. Do not resuspend the beads. Remove supernatant with a pipette.
 15. Repeat the 70% ethanol wash again by adding 400 μ L of 70% ethanol to the samples and let stand for 30-60 seconds. Do not resuspend the beads. Remove supernatant with a pipette.
 16. Place the tubes in the thermomixer and mix at 1000 rpm for 3-4 minutes to dry the beads. NOTE: IT IS IMPORTANT TO REMOVE ALL RESIDUAL ETHANOL BEFORE PROCEEDING TO THE NEXT STEP.
 17. Remove the tubes from the magnet and add 120 μ L of 10mM Tris to each sample.
 18. Place the tubes in the thermomixer and alternate mixing at:
 - i. 1000 rpm for 2-3 minutes
 - ii. Let stand for 2-3 minutes.
 - iii. 1000 rpm for 2-3 minutes.
 19. Place the tubes on the magnet for 10 minutes or wait for solution to clear.
 20. While the tubes are still on the magnet, transfer 110 μ L of the DNA solution to a clean tube.
 21. If beads are present visually in the DNA solution, place the sample back on the magnet. While the sample is still on the magnet, transfer 100 μ L of DNA to a clean tube. Cap the tube.
 22. DNA can be stored at 4–8°C for up to four weeks. For storage longer than four weeks, store the sample DNA in a –20° or –80°C non-frost-free freezer.

Table 5: Recommended Supplies and Equipment

Supplies and Equipment	Hologic Part Number
Cervista MTA System for automation users	PRD-01406
Conversion Solution (for use with SurePath Liquid Cytology cervical samples)	PRD-01457
2.2 mL deep well plates (Abgene/Fisher Scientific): BC-3082	LBS-00006
Aspirator and spacer (includes tubes, stoppers and connectors)	12-234
SPRI Plate 96R Super Magnet (Beckman Coulter /Fisher Scientific): NC9596962	12-238
SPRI Magnetic 6 Tube Stand (Beckman Coulter /Fisher Scientific): 001139	16-1000

Microplate Exchangeable Thermoblock (Eppendorf/Fisher Scientific): 05-400-35	12-239
Microplate Exchangeable Thermoblock, 2.0mL Tubes (Eppendorf/Fisher Scientific): 05-400-204	16-1001
Thermomixer w/ MTP Adapter (Eppendorf/Fisher Scientific):05-400-205	12-240 (115V)
Vacuum pump (Gast/Fisher Scientific): 01-092-29	12-241 (115V)
Vacuum pump (Gast/Fisher Scientific): 01-092-26	12-262 (230V)
Centrifuge, Benchtop (Thermo/Fisher Scientific): 75412452	LEQ-00002 (120V)
Centrifuge, Benchtop (Thermo/Fisher Scientific): 75004240	LEQ-00004 (230V)
Benchtop Centrifuge w/30 tube rotor (Eppendorf/Fisher Scientific): 022620509	NA
Benchtop Centrifuge w/30 tube rotor (Eppendorf/Fisher Scientific): 022620525	NA
VWR Digital Dry Block Heater 120 (12621-088)	16-005
VWR Modular Heating Block for Titer Plates (13259-295)	NA

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The Genfind DNA Extraction Kit utilizes SPRI paramagnetic bead technology and additional components, covered under U.S. Patent Nos. 5,705,628; 5,898,071; 6,534,262 and any corresponding international equivalents.

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