GenSolve™ DNA COMPLETE

GSC-50 & GSC-100

User Guide

DNA recovery and purification from Ahlstrom GenSaver™ cards ,FTA® paper, Guthrie Cards, GenPlate® elements and other papers

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GenSolve DNA Recovery

Quick Guide

Simple Recovery Protocol

Step 1

To the DBS element add Lysis solution, then add Proteinase K

Step 2

Incubate at 56°C for 1½ hours with agitation at 1400 rpm

Step 3

Transfer solution plus paper to Spin Basket then centrifuge. Add Recovery Solution B

Step 4

Proceed with DNA purification

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GenSolve DNA Purification

Quick Guide

Simple Purification Protocol

Step 1

Add 600 µl of 100% Ethanol to the extracted DNA solution, add to the purification column

Step 2

Wash column with Wash Solution 1

Step 3

Wash column with Wash Solution 2

Step 4

Elute DNA

Step 5

Concentrate DNA (Optional)

Overview

The GenSolve DNA Complete kit recovers double-stranded DNA from paper spotted with blood or other biological material, regardless of storage time, for subsequent purification. DNA can be efficiently recovered from treated paper (i.e. FTA, GenPlate elements, and GenSaver™ cards) and untreated paper (i.e. Guthrie cards, GenCollect™ cards). The FTA chemistry, available in a 384-well format as GenPlates, lyses blood cells upon contact and exposing the DNA, stabilizing it at room temperature.

The Protease component of GenSolve, coupled with lysis solution, enhances digestion of dried biological material when held at an elevated temperature, liberating bound DNA from the paper. The resulting lysate, which is a mixture of DNA, cellular debris, and FTA chemicals (if applicable) is then applied directly onto a purification column. Subsequent washing steps separate the DNA from cellular material. A final elution step results in purified DNA ready for most genetic analysis techniques.

Recovered DNA will be at a concentration too low to be measured by traditional spectrophotometry, i.e. NanoDrop. A fluorimeter method such as PicoGreen or quantitative PCR is recommended for quantitation.

Product specifications

GenSolve DNA Complete is designed to meet the following performance specifications when recovered from whole blood applied to 6 mm disks of paper.

Yield - The expected yield range is 50 to 350 ng/disk, depending on the white blood cell content of the original sample. The expected average yield is 130 ng/disk.

Concentration - The expected range is 0.5 to 2 ng/µL.

Size - The majority of fragments are at least 35 kb.

Reproducibility - <20% CV between triplicate samples and between assays.

Quantity - Expected range of purified DNA is 130 ng

PCR amplification - Successful amplification with primers used in both research and clinical laboratories.

The kit is intended for laboratory use only. Certain chemicals used in GenSolve reagents may be hazardous: lithium dodecyl sulfate, quanidinium-HCl.

Safety information

Certain chemicals present in FTA paper may be hazardous: sodium dodecyl sulfate, uric acid, and EDTA, (ethylenediaminetetraacetic acid). GenSolve DNA contains Protease K which is a sensitizer and an irritant. Care should be taken when working with hazardous chemicals, such as minimizing contact and wearing appropriate personal protective equipment (safety glasses, gloves and a lab coat). Avoid contact with eyes and ingestion.

Kit contents GSC-50

1. Lysis solution (LS); 62 mL, 1 bottles
2. Proteinase K solution; 0.6 mL, 1 vial, yellow cap
3. Recovery Solution B; 2.2 mL, 1 vial, white cap
4. Wash Solution 1 (WS1), 37.4 mL, 1 bottle ǂ
5. Wash Solution 2 (WS2), 10.5 mL, 1 bottle ǂǂ
6. Elution Buffer (EB), 8 mL, 1 bottle
7. Screw cap tubes, 1.5 mL 50 ea.
8. Spin Basket/2 ml Dolphin-Tube Assembly, 50 ea.
9. Dolphin tubes, 2.0 mL, 100 ea.
10. DNA columns, 50 ea.
11. User Guide

ǂ Add 16 ml of 100% Ethanol to WS1

ǂǂ Add 42 mL of 100% Ethanol to WS2

Note: See page nine for additional guidance

An additional 30 mL of 100% Ethanol is required for addition to individual samples.

Kit contents GSC-100

1. Lysis solution (LS); 62 mL, 1 bottles
2. Proteinase K solution; 0.6 mL, 2 vial, yellow cap
3. Recovery Solution B; 2.2 mL, 1 vial, white cap
4. Wash Solution 1 (WS1), 37.4 mL, 1 bottle ǂ
5. Wash Solution 2 (WS2), 10.5 mL, 1 bottle ǂǂ
6. Elution Buffer (EB), 8 mL, 1 bottle
7. Screw cap tubes, 1.5 mL 100 ea.
8. Spin Basket/2 ml Dolphin-Tube Assembly, 100 ea.
9. Dolphin tubes, 2.0 mL, 200 ea.
10. DNA columns, 100 ea.
11. User Guide

ǂ Add 16 ml of 100% Ethanol to WS1

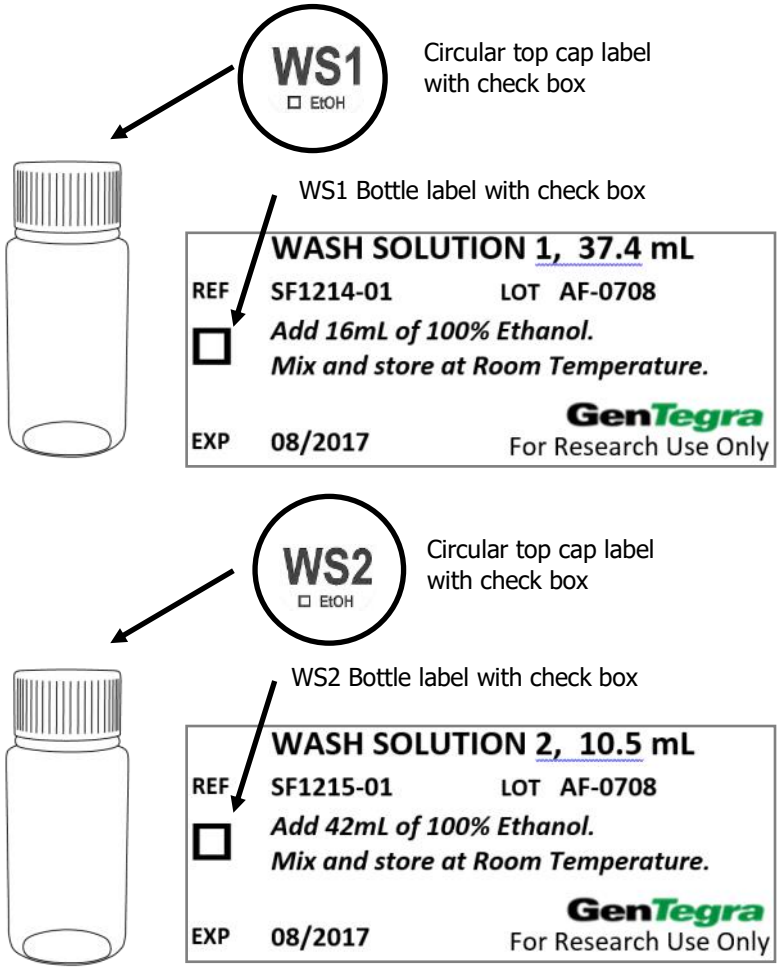
ǂǂ Add 42mL of 100% Ethanol to WS2

Note: See page nine for additional guidance

An additional 60 mL of 100% Ethanol is required for addition to individual samples.

Check boxes to prevent errors

Both wash solutions, WS1 and WS2, are provided with check boxes on the bottles labels. Use the check box to confirm that the 100% Ethanol has been added and the wash solution is complete and ready to use. After adding the 100% Ethanol check either or both boxes.



For use with GenSolve GSC-50 and GSC-100

GenPlates®, other FTA-based storage papers, Ahlstrom GenSaver™ cards and all other Dry Blood Spot (DBS) samples.

GenPlate GVN24P shown

Ahlstrom GenSaver™ card for DNA

Storage

Proteinase K should be stored at 2-8 °C upon arrival. It has a shelf life of 6-months from date of shipment.

All other components can be stored at either room temperature or 2-8 °C.

After addition of Protease K, Lysis Solution should be used within 2-3 hours for maximum DNA yield.

Additional equipment and materials required

- Incubator/Shaker (e.g. VorTemp™ 56 Shaking Incubator)
- Ethanol, 100% Molecular Biology Grade
- Microfuge Tubes
- Millipore Microcon MRCF0R100 (optional)
- P20, P200 and P1000 pipettes, pipette tips
- Microcentrifuge

GenSolve protocols

DNA recovery

Notes: The protocol can efficiently process GenPlate elements and 6 mm FTA or Guthrie card punches. For maximum DNA yield, ensure that spotted paper has been cured for at least two weeks. If higher concentrations or more DNA is desired, up to three elements (6 mm disks) can be processed simultaneously without changing protocol.

1. Pre-heat Incubator/Shaker to 56°C.

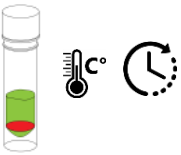
2. For each reaction combine 609uL of Lysis Solution with 11uL of Proteinase K in 1.5 ml screw top tube

3. Close cap and vortex briefly to mix Solution (Stores at RT for 2-3hrs)

Note: After addition of Protease, Lysis Solution should be used within 2-3 hours for maximum recovery.

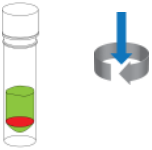
4. Punch up to three 6mm DBS elements into a 1.5mL screw top tube containing DBS Lysis Solution + Proteinase K

5. Close cap and place on Incubator/vortex Shaker and shake at 1400 RPM for 1.5 hr at 56°C



After removing the samples from shaker, place 50uL of elution buffer per sample at 56°C for later use in DNA purification at (Step 10)

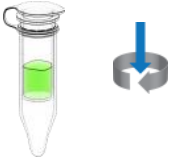
6. Remove samples from shaker, vortex samples for 5 sec then briefly centrifuge



7. Place spin baskets in 2mL dolphin tubes

8. Decant DBS and Lysis Solution mixture into spin basket

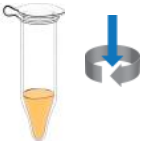
9. Close cap and centrifuge at 16,300 x g or greater for two minutes. (Discard spin basket and element)



10. To filtrate in 2.0 ml dolphin tube add 20uL of Recovery Solution B per sample

11. Add 600uL of 100% Ethanol to each sample

12. Close cap and vortex for 5 sec then briefly centrifuge



13. The DNA solution is now ready for the DNA purification protocol

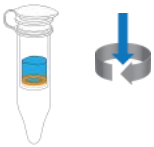
Proceed directly to DNA purification on next page.

DNA purification

1. Place DNA column in dolphin tube

2. Transfer 600 uL of DNA solution from step 13 on the previous page to the DNA column

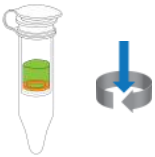
3. Close cap and spin down at 6000 rcf for 30 sec , discard filtrate



4. Repeat steps 2 & 3 until all sample has been loaded on the column

5. Add 500uL of WS1* to DNA column

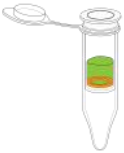
6. Close cap and spin down at 6000 rcf for 30 sec, discard filtrate



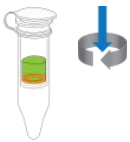
* Note: make certain that 100% ethanol has been added to Wash Solution 1

7. Place DNA column in new dolphin tube

8. Add 500 uL of WS2* to DNA column



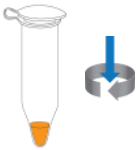
9. Close cap and spin tube and column at 8000 RPM for 30sec, discard filtrate. Place tube and column in centrifuge again and spin at max speed for 2min to dry column



10. Place spin column in new 2mL dolphin tube and add 50uL of Elution buffer (@ 56°C)

11. Incubate for 1min at RT

12. Spin columns at max speed for 2 min, discard column.



13. The eluate is ready for quantitation and downstream analysis. To concentrate DNA, proceed to protocol on next page.

* Note: make certain that 100% ethanol has been added to Wash Solution 2

DNA concentration (Optional)

This addendum will assist in concentrating recovered DNA. Note: protocol requires Millipore Microcon YM- 100 (Millipore #42413) columns.

Do not allow Microcon membrane to dry with sample on it. Do not touch the membrane with pipet tip during sample addition or wash steps. When washing and concentrating gDNA samples, do not spin at more than 500xg (2400 rpm).

1. Insert MICROCON-YM-100 sample reservoir into a microfuge tube.
2. Add 50 µL of water and spin at 12,300 rpm (14,000 x g) for 3 minutes.
3. Apply up to 500 µL of SAMPLE onto MICROCON-YM-100.
4. Spin 2,400 rpm (500 x g) for 15 minutes (LOW SPEED SPIN).
5. Decant microfuge tube and repeat steps 3 and 4 until the entire sample has been applied.
6. Transfer MICROCON-YM-100 to a new microtube.
7. Add 250 µL of water to MICROCON-YM-100.
8. Spin at 2,400 rpm for 15 minutes (LOW SPEED SPIN).
9. Optional: decant microcentrifuge tube and repeat steps 7-8.

10. Continue to centrifuge until ~25µl remains on the column. For maximum recovery, do not spin to dryness. If processing multiple MICROCON-YM-100 units, independently monitor each unit, as they concentrate at different rates.

11. Gently pipette mix the ~25 µL being careful not to touch the filter at the bottom of the filtration unit.

12. Invert MICROCON-YM-100 and transfer into a new microcentrifuge tube.

13. Spin at 3,500 rpm (1,100 x g) for 3 minutes to collect the DNA.

Notes on the age of DBS samples

Overview

The age of a DBS has a marked affect on the recovery of DNA and how much agitation is required to provide optimum recovery of DNA. DBS samples that are less than 2-years old require much more vigorous mixing to provide the expected yield of DNA. As most DBS samples are archived for longer than two years before use our standard extraction protocol is for older (> 2-years old) samples.

DBS samples greater than 2-years old

The protocol details provide in step & of page 9 are those for older DBS. If fraying of the paper disks is noted then recoveries should be good.

DBS samples less than 2-years old

DBS samples that are less than 2-years old require more vigorous agitation and the paper matrix needs to be fully disrupted to ensure good yields of DNA. These fresher samples are harder to extract because the proteins in conjunction with the paper matrix act as a glue/shield to the GenSolve reagents and prevent access to the DNA. Strong mechanical agitation or vigorous vortexing every 5-minutes may be necessary to completely disrupt the paper matrix. Cutting the paper disk into very small pieces before extraction may also improve DNA extraction.

These extra mechanical efforts are not harmful to the quality of the DNA and are not required for older DBS samples. We do not recommend extra or prolonged heating to improve extraction as it will affect the quality of the DNA.

Frequently asked questions (FAQs)

How can I convert RPM to g (rcf)?

Use the correct centrifugation speeds in order to maximize yield and purity. Check your centrifuge setting using the following equation:

g(rcf) = 1.12*r*(rpm/1000), where r is the radius of the rotor in mm.

I left my Proteinase K out at room temperature. Can I still use it?

Yes, up to 24 hours. Store the proteinase at 4°C. Refer to page 9 for additional storage information.

How do I get a material safety data sheet (MSDS)?

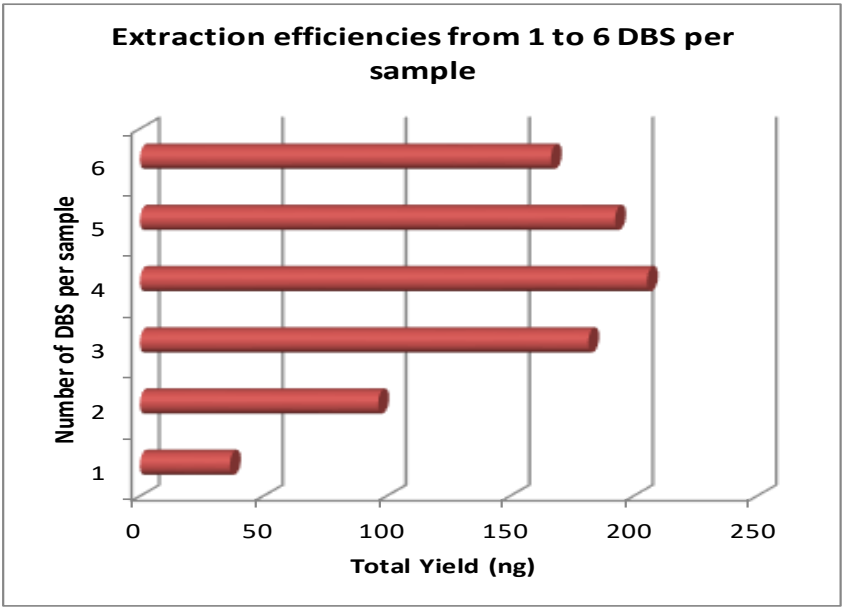
MSDS documents are available by contacting Technical Support at support@gentegra.com.

Where can I find further information about DNA quantitation and setting up a PicoGreen assay?

Refer to PicoGreen Assay Protocol provided by supplier.

My discs are not 6 mm, how many can I use?

A 6 mm disc is ($\pi * r^2$) or 28.27 mm^2, whatever the size of your paper discs the total area of paper used should not exceed 85 mm^2 for maximum efficiency. See chart below.



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