GenTegra GenSolve Classic User Guide

GVR-113

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For Research Use Only

Whole blood DNA recovery from Ahlstrom-Munksjö GenSaver Card, FTA® paper, Guthrie Cards, GenPlates® elements and other paper

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Simple Recovery Protocol

Step 1

To the Lysis Solution (LS), add Proteinase K, mix, then add to paper in tube

Step 2

Incubate at 56 °C for one hour with agitation

Step 3

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

Step 4

Proceed with DNA purification

For use with GenSolve GVR113

GenPlates, untreated filter paper and other FTA-based storage mediums

GenPlate GVN24P shown

Ahlstrom GenSaver™ card for DNA

Overview

The GenSolve kit recovers double-stranded DNA from paper spotted with blood or other biological material, regardless of storage time, for subsequent purification via a Blood DNA purification Kit. GenSolve extraction chemistry is also available as part of GenSolve COMPLETE DNA extraction and purification Kit. DNA can be efficiently recovered from treated paper (i.e. GenSaver™ and FTA) 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 binds DNA, stabilizing it over the long term at room temperature.

The Proteinase component of GenSolve, coupled with recovery 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 membrane. 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. A fluorimeter method such as PicoGreen or quantitative PCR is recommended for quantitation.

Product specifications

GenSolve was designed to meet the following performance specifications when recovered from whole blood applied to 6 mm disks of FTA® paper (i.e. GenPlate elements).

Yield - The expected yield range from FTA paper 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.

If using GenSaver paper the expected yields are 100-700 ng/disk which represents >95% recovery of the DNA on the card.

Concentration - The expected range is 0.5 to 4 ng/µL.

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

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

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.

Safety information

Certain chemicals present in FTA paper may be hazardous: sodium dodecyl sulfate, uric acid, and ethylene-diaminetetraacetic acid. 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 (For Trial kits see page 18.)

1. Lysis solution (LS); 62 mL, 1 bottle
2. Proteinase K solution; 0.6 mL, 2 vials, yellow cap
3. Recovery Solution B; 2.2 mL, 1 vial, white cap
4. User Guide

Kit Storage

Proteinase K should be stored at 2-8°C upon arrival.
All other components can be stored at room temperature.

Additional equipment and materials required

- The **Thermomixer Compact** model thermal shaker is highly recommended to ensure proper vigorous shaking is provided. It is the one we use in our laboratory.
- 1.5 mL Screw Cap tubes & Caps
- Spin Basket/Tube Assembly (GenTegra #GVSPIN200) or equivalent.
- Ethanol, 100%
- GenTegra DNA Purification Kit or Qiagen QIAamp DNA Blood Mini Kit
- 2.0 ml collection tubes, dolphin or Qiagen
- Millipore Microcon MRCF0R100 (optional)
- Microcentrifugefuge Tubes
- P200 and P1000 pipettes, pipette tips
- Microcentrifuge

GenSolve protocol: DNA recovery

Notes: This protocol can efficiently process GenPlate elements and 6 mm punches from GenSaver, GenCollect, FTA or Guthrie cards. 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. Do not use more than 3 punches per sample, see page 17.

1. Pre-heat Incubator/Shaker to 56°C.

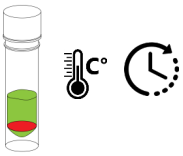
2. For each reaction combine 609 µL of Lysis Solution with 11 µL of Proteinase K in 1.5 or 2mL screw cap or flip-top dolphin tube. (User supplied.)

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

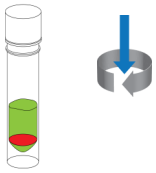
Note: After addition of Protease, Lysis Solution should be used within 2-3 hours for maximum recovery.

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



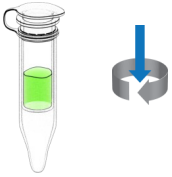
6. Remove samples from shaker, vortex samples for 5 sec then briefly centrifuge



7. Place spin baskets in 2mL dolphin tubes (Hint: Transfer the majority of lysis supernatant to the dolphin tube before placing spin basket in dolphin tube.)

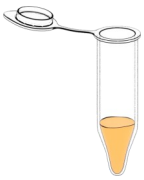
8. Decant DBS and Lysis Solution mixture into spin basket

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

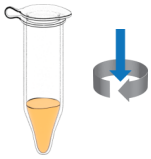


10. To filtrate in 2.0 ml dolphin tube add 20uL of Recovery Solution B per sample

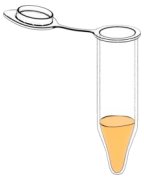
11. Add 600uL of 100% Ethanol per sample



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



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



DNA Purification

Proceed directly to DNA purification protocol.

a. See GenSolve DNA COMPLETE User Guide

b. See User Guide for the purification kit you are using

DNA concentration

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 500 xg (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.

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.

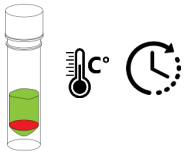
	Standard protocol*	Age modified Protocol		
Age of DBS Years)	≤2	2-4	5-7	≥8
Lysis incubation @ 56°C (hours)**	0	2-3	4-6	8-10
Proteinase K treatment @ 56°C	1.5	1.5	2	2

* Standard protocol, see page 8

** Separate Lysis incubation before adding Proteinase K is not recommended for standard protocol

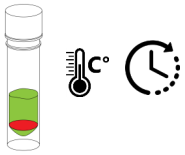
2. For each sample tube add 609uL of Lysis Solution followed by up to three 6mm DBS elements into a 1.5mL screw top tube

3. Close cap and vortex briefly to mix Lysis Solution and punches, place on Incubator/vortex Shaker and shake at 1400 RPM for time in table above at 56°C.



4. Add 11uL of Protease K to Lysis Solution and punches.

5. Close cap and place on Incubator/vortex Shaker and shake at 1400 RPM for time in table above at 56°C



Continue with step 6 on page 9.

Frequently asked questions

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.

You recommend the Eppendorf Thermomixer Compact is this specific model important or will any thermal shaker work as well?

The application of vigorous shaking is important if freeing the DNA from the paper matrix. Without vigorous shaking the quantity of DNA extracted can be lower than what our reported values. The Thermomixer Compact is the model we use routinely in our testing as it provides the required level of vigorous shaking to ensure proper DNA extraction.

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

Refer to PicoGreen Assay Protocol provided by supplier.

This GenSolve kit looks different from the one I used before, have you changed something?

Yes, we have simplified the kit by combining two reagents into one and in so doing simplified the process. Performance and shelf life remain unchanged.

You recommend a VorTemp™ 56 Shaking Incubator, is this important?

Yes, very important, this shaker is capable of providing the needed vigorous agitation to ensure proper DNA extraction.

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

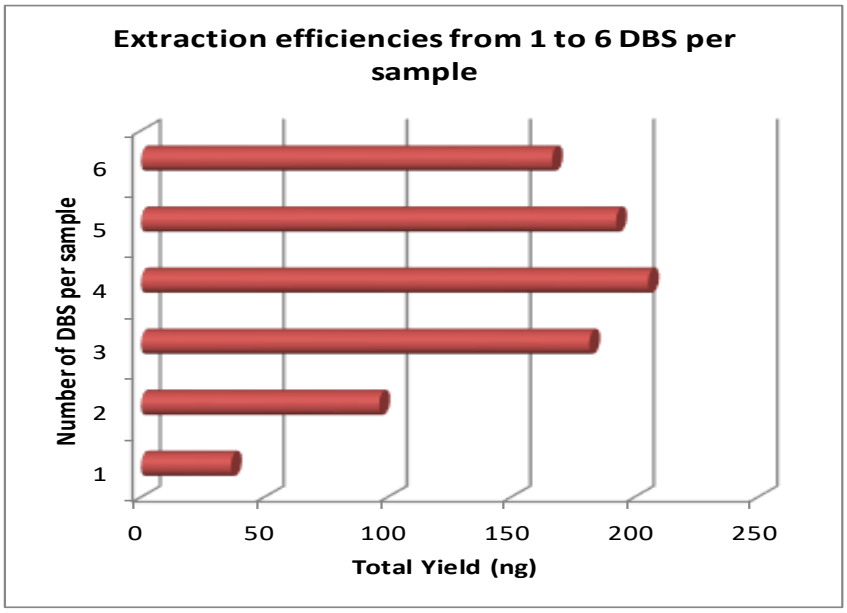
Yes, up to 24 hours. Store the protease 4°C. Refer to page 6 for additional storage information.

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

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

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.



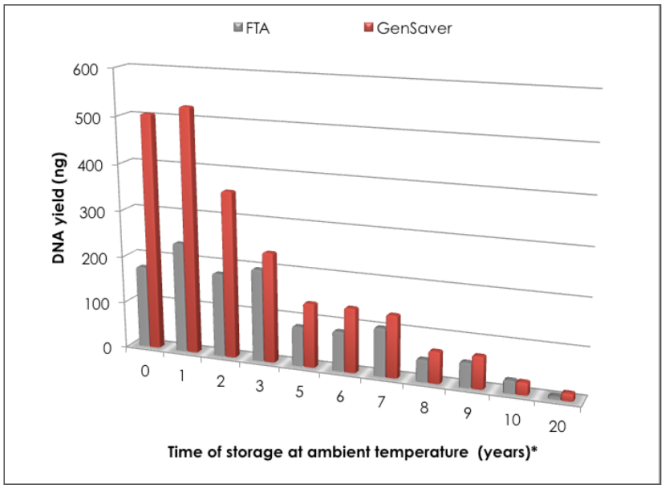
Data in this figure is for extraction of DNA from FTA Classic paper or Whatman 903 paper.

I am using GenSaver paper how much can I use for optimum DNA extraction?

Optimum yield is obtained by using ¼ of the circle for a single extraction. This is 123

My old DBS spots seem to lost much of the DNA after 5 or 10 years, is it really gone?

Fortunately the answer is no, your DNA is not lost it is only harder to extract. Over time the protein and cell debris that make up the DBS spot make it harder and harder to free the DNA from this matrix. The plot below shows how the amount of DNA drops if the same extraction protocol is followed for samples as they age.



Data in above figure used the same blood sample for all DBS.

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