GenTegra GenSolve™ **DNA COMPLETE**

GSC-50B & GSC-100B

User Guide

DNA recovery and purification from
Dried Blood Spots
Ahlstrom GenSaver™ cards ,FTA® paper,
Guthrie Cards, GenPlate® elements
and all other papers with DBS

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For Research Use Only

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

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Proceed with DNA purification

GenSolve DNA Purification

Quick Guide

Simple Purification Protocol

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

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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 all genetic analysis techniques.

Recovered DNA may 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.

Next Generation Sequencing—Successful whole genome

This kit is intended for Research Use Only.

Safety information

Certain chemicals used in this kit and/or in FTA paper may be hazardous, including lithium dodecyl sulfate, sodium dodecyl sulfate, guanidinium-thiocyanate, uric acid, proteinase K and EDTA(ethylenediaminetetraacetic acid). Protease K 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-50B

1. Lysis solution (LS); 62 mL, 1 bottles
2. Proteinase K solution; 0.6 mL, 1 flip top dolphin tube
3. Recovery Solution B; 2.2 mL, 1 vial, white cap
4. Wash Solution 1 (WS1), 30 mL, 1 bottle ǂ
5. Wash Solution 2 (WS2), 18 mL, 1 bottle ǂ
6. Elution Buffer (EB), 25 mL, 1 bottle
7. Dolphin tubes, flip top, 1.5 mL 50 ea.
8. Spin Basket/2 ml Dolphin-Tube Assembly, 50 ea.
9. Collection tubes, 2.0 mL, 150 ea.
10. DNA columns, 50 ea.
11. User Guide

ǂ Add 30 ml of 100% Isopropyl alcohol (IPA) to complete WS1

ǂǂ Add 42 mL of 100% Isopropyl alcohol (IPA) to complete WS2

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

Molecular grade IPA and Ethanol are not included in the contents of the kit.

Kit contents GSC-100B

1. Lysis solution (LS); 62 mL, 1 bottles
2. Proteinase K solution; 0.6 mL in each of 2 flip top dolphin tube
3. Recovery Solution B; 2.2 mL, 1 vial, white cap
4. Wash Solution 1 (WS1), 30 mL, 1 bottle ǂ
5. Wash Solution 2 (WS2), 18 mL, 1 bottle ǂ
6. Elution Buffer (EB), 25 mL, 1 bottle
7. Dolphin tubes, flip top, 1.5 mL 100 ea.
8. Spin Basket/2 ml Dolphin-Tube Assembly, 100 ea.
9. Collection tubes, 2.0 mL, 300 ea.
10. DNA columns, 100 ea.
11. User Guide

ǂ Add 30 ml of 100% Isopropyl alcohol (IPA) to complete WS1

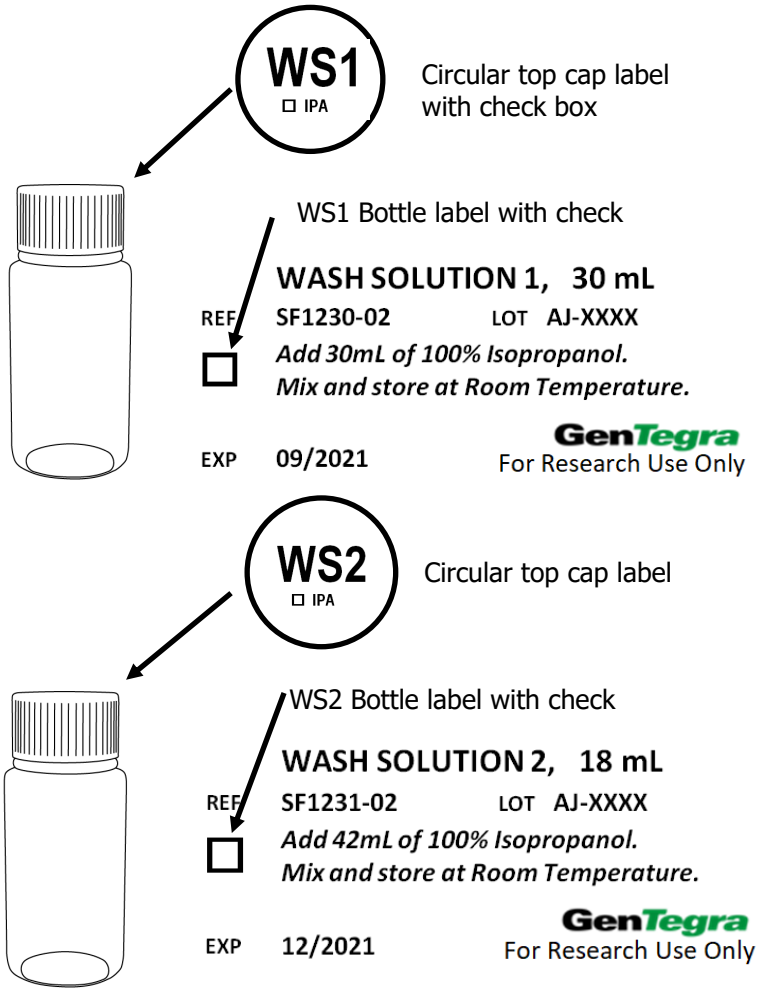
ǂǂ Add 42 mL of 100% Isopropyl alcohol (IPA) to complete WS2

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

Molecular grade IPA and Ethanol are not included in the contents of the kit.

Check boxes to prevent errors

Wash solutions WS1 and WS2, are provided with check boxes on the bottles labels. Use the check box to confirm that the 100% IPA has been added and the wash solution is complete and ready to use. After adding the 100% IPA check either or both boxes. After IPA addition each wash solution is stable for one year at room temperature (i.e. 25°C).



For use with GenSolve GSC-50B and GSC-100B

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

GenPlate GVN24P shown

Ahlstrom-Munksjö 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

- 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.
- Ethanol, 100% Molecular Biology Grade
- Isopropanol, 100% Molecular Biology Grade
- Microcentrifuge Tubes
- Millipore Microcon MRCF0R100 (optional)
- P20, P200 and P1000 pipettes, pipette tips
- Microcentrifuge

GenSolve protocols: 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, see page 22.

Note: Pre-heat Incubator/Shaker to 56°C.

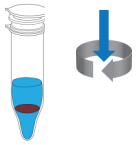
1. For each reaction combine 609 µL of Lysis Solution with 11 µL of Proteinase K in 2 ml screw cap dolphin tube.

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

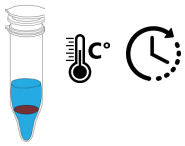
Note: After addition of Protease, Lysis Solution should be used within 2-3 hours for maximum recovery.

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

5. Close cap and pulse vortex the samples for 5 to 10 seconds.



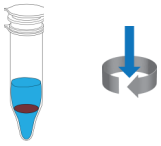
6. Centrifuge samples for 2 minutes at top speed to make any drops come sown from the lid and facilitate the contact between the sample and lysis solution.



7. Place on Incubator/vortex Shaker and shake at 1400 rpm for 1.5 hr. at 56°C.

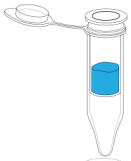
8. After removing the samples from shaker, preheat 140 µL of elution buffer per sample in 56°C bath for later use in DNA purification at (Step 10, page 17).

9. Remove sample tubes from shaker, vortex samples for 5 sec then centrifuge at top speed for 2 to 5 min to get rid of bubbles.

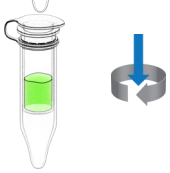


10. Place spin baskets in 2 mL snap cap dolphin tubes. (Hint: Transfer the majority of lysis supernatant to the dolphin tube before placing spin basket in dolphin tube.)

11. Use pipette to transfer the DBS and then pipette the remaining mixture into the spin basket.



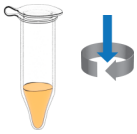
12. Close cap and centrifuge at 16,300 x g or greater for two minutes. (Discard spin basket and element)



13. Add 20uL of Recovery Solution B to filtrate in each 2.0mL dolphin tube.

14. Add 600µL of 100% Ethanol to each sample.

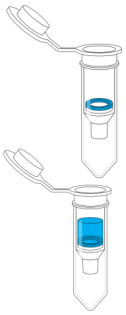
15. Close cap and vortex for 5 sec then briefly centrifuge.



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

Proceed directly to DNA purification on next page.

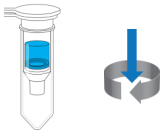
DNA purification



1. Place DNA column in a fresh collection tube.

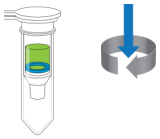
2. Transfer 600 µL of DNA solution from step 16 on the previous page to the DNA column.

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



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

5. Add 500uL of Wash 1 to DNA column.*



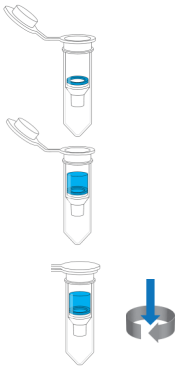
6. Close cap and spin down at 6000 x g for 30 sec, discard filtrate and collection tube.

* **Note:** Make certain that 100% IPA has already been added to both Wash Solution 1.

7. Place DNA column in new collection tube.

8. Add 500 µL of WS2* to DNA column.

9. Close cap and spin tube and column at 6000 x g for 3 minutes, discard filtrate. Place tube and column in centrifuge again and spin 16,000 x g for 1 min. to dry column.



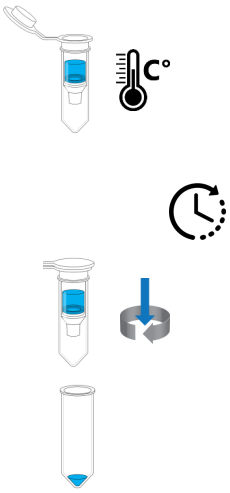
Note: Do not deviate from centrifuge times and speeds, over drying the column can lead to decreased DNA yield.

10. Place spin column in new 2mL collection tube and add 100 µL of 56°C Elution buffer. Discard old tube.

11. Incubate for 1 min at Room Temperature (21-25°C).

12. Spin columns at 6,000 x g for 1 min, discard column.

13. Transfer eluent to an RNase/DNase free flip-cap or screw cap tube. The eluate is ready for quantitation and downstream analysis. To concentrate DNA, proceed to protocol on next page.



* **Note:** Make certain that 100% IPA has already been added to both 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 500 x g (2400 rpm).

1. Insert MICROCON-YM-100 sample reservoir into a microfuge tube.
2. Add 50 µL of water and spin at 14,000 x g for 3 minutes.
3. Apply up to 500 µL of SAMPLE onto MICROCON-YM-100.
4. Spin 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 500 x g 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 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 on pages 13-15 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.

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, however, any thermal shaker that provides the required level of vigorous shaking can be used for a proper DNA extraction.

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)?

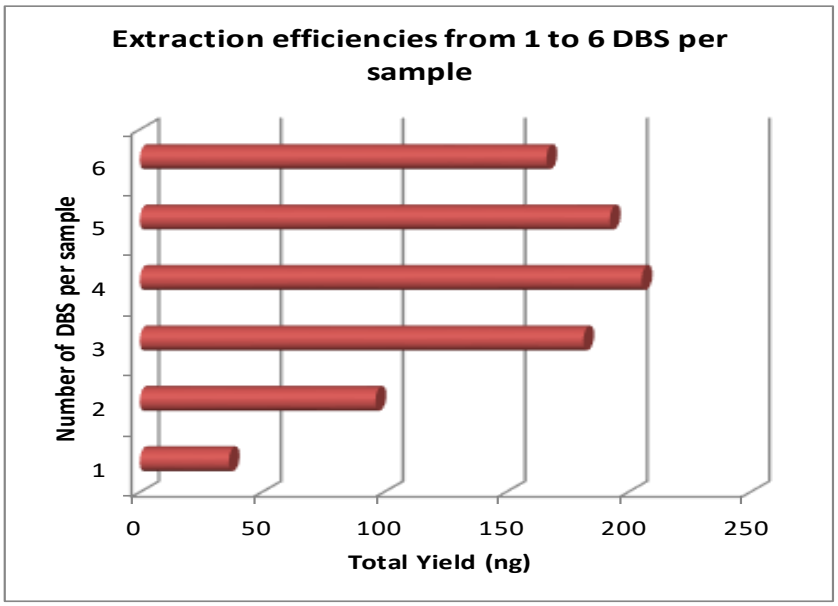
MSDS documents are available by contacting Technical Support at support@gentegra.com.

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

Refer to PicoGreen Assay Protocol provided by supplier.

My FTA Classic 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. Three 6 mm discs is optimum. See chart below. Optimum is defined as the most DNA re-covered form each disc but the maximum amount of total DNA recovered is using 4 discs.



Data in this figure is for extraction of DNA from FTA Classic paper or Whatman 903. Quantity of DNA obtained will be 2X if using GenSaver 1.0 or 2.0 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 mm^2 or four 6 mm discs.

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GenTegra products are for research use only.

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For warranty and liability information, please see GenTegra terms and conditions of sale.

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