User-Developed Protocol:

For purification of DNA from stool samples for pathogen detection using the BioSprint 15 DNA Blood Kit

This protocol has been adapted by customers and is for purification of DNA from stool samples using the BioSprint 15 DNA Blood Kit in combination with the BioSprint 15 workstation. The lysis conditions used in this protocol result in an increased ratio of non-host DNA to host DNA. Host DNA is not excluded by this protocol. **The procedure has not been thoroughly tested and optimized by QIAGEN.**

Introduction

The BioSprint 15 DNA Blood Kit uses MagAttract® magnetic-particle technology for purification of highly pure DNA. MagAttract technology combines the speed and efficiency of silica-based nucleic acid purification with the convenient handling of magnetic particles and enables purification of high-quality DNA that is free of proteins, nucleases, and other impurities. Purified DNA is suitable for direct use in downstream applications, such as amplification or other enzymatic reactions.

This protocol describes first the lysis of stool samples. After lysis, DNA-damaging substances and PCR inhibitors present in the stool sample are adsorbed to the InhibitEX® matrix and removed by centrifugation. DNA is then purified using a fully automated procedure on the BioSprint 15.
This protocol is not for use in diagnostic procedures.

IMPORTANT: Please read the *BioSprint DNA Blood Handbook*, paying careful attention to the "Safety Information" and "Important Notes" sections, before beginning this procedure. Ensure that you are familiar with operating the BioSprint 15. See the *BioSprint 15 User Manual*.

Starting material

This protocol is for processing 180–220 mg fresh or frozen stool, but can also be used with larger amounts of stool. Starting with larger amounts of stool is recommended when the target DNA is not distributed homogeneously throughout the stool and/or is at a low concentration; a larger amount of starting material will increase the likelihood of purifying DNA from low-titer sources in stool samples. This protocol can also be used for samples of less than 180 mg. In this case, the amounts of buffers and other reagents must be reduced proportionally. We recommend using frozen stool as starting material.

Equipment and reagents to be supplied by the user

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, please consult the appropriate material safety data sheets (MSDSs). These are available online in convenient and compact PDF format at www.qiagen.com/ts/msds.asp where you can find, view, and print the MSDS for each QIAGEN® kit and kit component.

- BioSprint 15 workstation, cat. no. 9000850

- "BS15 DNA Blood 200" protocol; available from QIAGEN Technical Services or your local distributor
- BioSprint 15 DNA Blood Kit (45), cat. no. 940014, or BioSprint 15 DNA Blood Kit (360), cat. no. 940017
- Buffer ASL, cat. no. 19076
- QIAGEN Proteinase K (2 ml), cat. no. 19131, or QIAGEN Proteinase K (10 ml), cat. no. 19133
- Ethanol (96–100%)*
- InhibitEX tablets (for more information, please contact your local QIAGEN Technical Service Department)
- Isopropanol
- Vortexer
- Microcentrifuge tubes (2 ml); the tubes used in step 5 of the procedure should be wide enough to accommodate an InhibitEX tablet (e.g., Eppendorf® Safe-Lock, cat. no. 0030 120.094 or Sarstedt Safe-Seal, cat. no. 72.695)†
- Microcentrifuge tubes (1.5 ml)
- Microcentrifuge
- Shaker–incubator (e.g., Eppendorf Thermomixer Comfort, cat. no. 5355 000.011 with adapter for 2 ml microcentrifuge tubes, cat. no. 5362 000.019)†
- Pipettors and sterile, RNase-free pipet tips with aerosol barriers (20–1000 µl)
- Multidispenser (e.g., Finnpipette® Stepper from Thermo Electron see www.thermo.com)†
- Tubes for storage of purified DNA
- Soft cloth or tissue and 70% ethanol or other disinfectant to clean worktable
- Disposable gloves

Important points before starting

- Check that Buffer AW1 and Buffer AW2 have been prepared according to the instructions in the "Preparing reagents" section of the *BioSprint DNA Blood Handbook*.
- Check that Buffer AL and Buffer ASL do not contain a white precipitate. If necessary, incubate Buffer AL and Buffer ASL for 30 minutes at 37ºC with occasional shaking to dissolve precipitate.
- Ensure that you are familiar with operating the BioSprint 15. Refer to the *BioSprint 15 User Manual* for operating instructions.

* Do not use denatured alcohol, which contains other substances such as methanol or methyethylketone.
† This is not a complete list of suppliers and does not include many important vendors of biological supplies.

- All centrifugation steps should be carried out at room temperature (15–25°C) at 20,000 x *g* (approximately 14,000 rpm). Increase the centrifugation time proportionately if your centrifuge cannot provide 20,000 x *g* (e.g., instead of centrifuging for 5 minutes at 20,000 x *g*, centrifuge for 10 minutes at 10,000 x *g*).
- To increase robustness of downstream PCR assays of DNA eluates from stool samples, we strongly recommend adding BSA to PCR mixtures to a final concentration of 0.1 µg/µl (e.g., Serva, cat. no. 11920 or New England Biolabs® BSA, cat. no. BSA-007). To increase PCR specificity, we recommend the use of QIAGEN® HotStarTaq® *Plus* DNA Polymerase.

Things to do before starting

- Set a shaker–incubator with an adapter for 2 ml microcentrifuge tubes to 70°C for use in steps 3 and 12.
- Prepare a master mix according to the table below for use in step 20 of the protocol. Before adding MagAttract Suspension G, ensure that it is fully resuspended. Vortex for 3 minutes before using for the first time, and for 1 minute before subsequent uses.

  Note: Prepare a volume of master mix 10% greater than that required for the total number of sample purifications to be performed.

Reagent	Volume per reaction (µl)
Isopropanol	200
MagAttract Suspension G	30

Procedure

1. **Weigh 180–220 mg stool in a 2 ml microcentrifuge tube (not provided) and place the tube on ice.**

   If the sample is liquid, pipet 200 µl into the microcentrifuge tube. Cut the end of the pipet tip to make pipetting easier.

   If the sample is frozen, use a scalpel or spatula to scrape bits of stool into a 2 ml microcentrifuge tube on ice.

   Note: When using frozen stool samples, take care that the samples do not thaw until Buffer ASL is added in step 2 to lyse the sample; otherwise the DNA in the sample may degrade. After addition of Buffer ASL, all further steps can be performed at room temperature (15–25°C).

2. **Add 1.6 ml Buffer ASL to each stool sample. Vortex continuously for 1 min or until the stool sample is thoroughly homogenized.**

   Note: It is important to vortex the samples thoroughly. This helps ensure maximum DNA concentration in the final eluate.

3. **Heat the suspension for 5 min at 70°C.**

   This heating step increases total DNA yield 3- to 5-fold and helps to lyse bacteria and other parasites. The lysis temperature can be increased to 95°C for cells that are difficult to lyse (such as Gram-positive bacteria).

4. **Centrifuge the sample at 20,000 x *g* for 1 min to pellet stool particles.**
5. **Pipet 1.4 ml of the supernatant into a new 2 ml microcentrifuge tube (not provided) and discard the pellet.**

   Note: The 2 ml tubes used should be wide enough to accommodate an InhibitEX tablet (e.g., Eppendorf Safe-Lock, cat. no. 0030 120.094 or Sarstedt Safe-Seal, cat. no. 72.695). Transfer of small quantities of pellet material will not affect the procedure.
6. **Add 1 InhibitEX tablet to each sample and vortex immediately and continuously for 1 min or until the tablet is completely suspended. Incubate the suspension for 1 min at room temperature to allow inhibitors to adsorb to the InhibitEX matrix.**
7. **Centrifuge the sample at 20,000 x *g* for 3 min to pellet stool particles and inhibitors bound to InhibitEX.**

   Note: If processing more than 12 samples, we recommend centrifuging the samples in batches of no more than 12 samples each and then immediately proceeding to step 8. The pellets formed after centrifugation will break up if the supernatant is not removed immediately.
8. **Immediately after the microcentrifuge stops, pipet the supernatant into a new 1.5 ml microcentrifuge tube (not provided) and discard the pellet. Centrifuge the sample at 20,000 x *g* for 3 min.**

   Transfer of small quantities of pellet material from step 7 will not affect the procedure.
9. **Pipet 25 µl proteinase K into a new 2 ml microcentrifuge tube (not provided).**
10. **Pipet 200 µl supernatant from step 8 into the 2 ml microcentrifuge tube containing proteinase K.**
11. **Add 200 µl Buffer AL and vortex for 15 s.**

    Note: Do not add proteinase K directly to Buffer AL.

    It is essential that the sample and Buffer AL are thoroughly mixed to form a homogeneous solution.
12. **Incubate at 70°C for 10 min.**

    Optional: Centrifuge briefly to remove drops from the inside of the tube lid.
13. **During the incubation described in step 12, switch on the BioSprint 15 at the power switch.**
14. **Open the front door of the BioSprint 15 and slide out the tube strip tray.**
15. **Load up to fifteen 5-tube strips into the tube strip tray. One 5-tube strip is used per sample.**

    If loading five 5-tube strips or fewer, we recommend loading them as a single column. If loading ten 5-tube strips or fewer, we recommend loading them as 2 columns.

    Load the 5-tube strips in the tube strip tray so that the tab of each 5-tube strip faces to the left. Make sure that the 5-tube strips are fully inserted into the tray and are not skewed.

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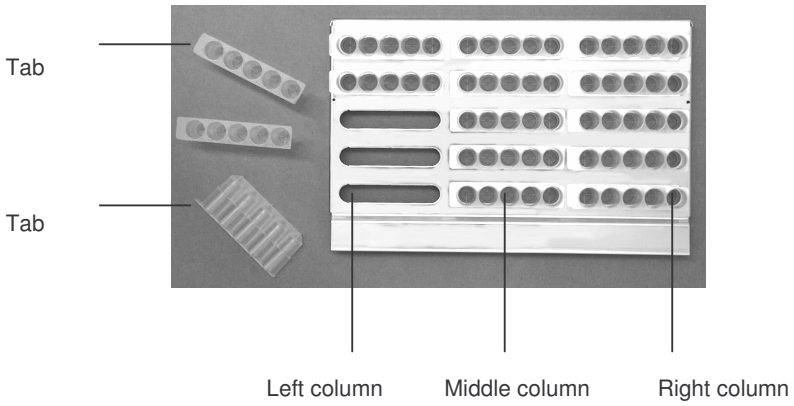
Correct Loading of 5-Tube Strips in the Tube Strip Tray



16. Add reagents into each 5-tube strip according to the table below.

Well	Reagent	Volume to add per well (µl)
1	Lysate*	630
2	Buffer AW1	700
3	Buffer AW2	500
4	Buffer AW2	500
5	Buffer AE	200

** Added at steps 19 and 20.*

Note: Well 1 is at the left of the 5-tube strip, well 5 is at the right.

Note: If processing more than one sample, record in which 5-tube strips you load the samples.

17. Load up to three 5-rod covers into the rod cover slots. There must always be a 5-rod cover above a column of 5-tube strips.

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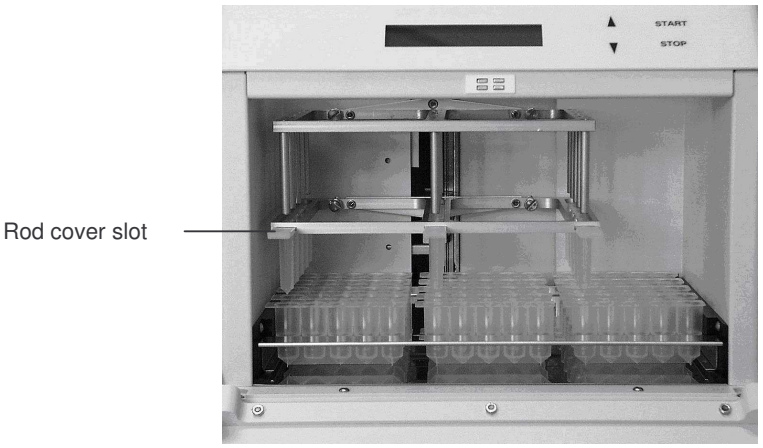
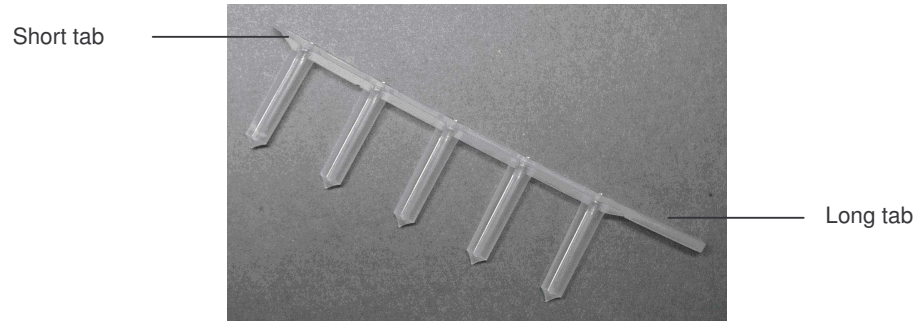
Rod Cover Slot



Insert a 5-rod cover into a rod cover slot so that the short tab faces inward and the long tab faces outward. 5-rod covers must be inserted so that they click into place.

Tabs of the 5-Rod Cover



IMPORTANT: Do not push 5-rod covers further after they click into place, otherwise an instrument crash will occur.

18. **Briefly centrifuge the 2 ml microcentrifuge tube to remove drops from the inside of the lid.**
19. **Carefully transfer 400 µl of the lysate to well 1 of the 5-tube strip.**
20. **Vortex the master mix containing isopropanol and MagAttract Suspension G (see "Things to do before starting") for 1 min. Add 230 µl master mix to each sample in well 1 of each 5-tube strip.**

    Note: If using a multidispenser, add 225 µl master mix to each sample.
21. **Slide the tube strip tray fully into the BioSprint 15.**

22. **Close the front door of the BioSprint 15.**

    Closing the front and top doors protects the samples from contamination.

23. **Select the protocol "BS15 DNA Blood 200" using the ▲ and ▼ keys on the BioSprint 15 workstation. Press "START" to start the protocol run.**

    Warning: Avoid contact with moving parts during operation of the BioSprint 15. See the *BioSprint 15 User Manual* for safety information.

24. **After the protocol run ends, press "STOP" and slide out the tube strip tray. Transfer the eluted DNA from well 5 of each 5-tube strip to other tubes for long-term storage.**

    Note: Well 5 is at the right of the 5-tube strip.

    Carryover of magnetic particles in eluates will not affect most downstream applications. If the risk of magnetic-particle carryover needs to be minimized, tubes containing eluate should first be placed in a suitable magnet and the eluates transferred to clean tubes (see the appendix of the *BioSprint DNA Blood Handbook*).

    Note: When using eluates in PCR, we strongly recommend adding BSA to a final concentration of 0.1 µg/µl to the PCR mixture. For maximum PCR specificity, we recommend using QIAGEN HotStarTaq *Plus* DNA Polymerase (visit www.qiagen.com for more information). For best results in downstream PCR analyses, use the minimum amount of eluate possible; the volume of eluate used as template should not exceed 10% of the final volume of the PCR. Large amounts of template DNA may inhibit PCR.

25. **Remove the 5-tube strips and 5-rod covers and discard them according to your local safety regulations.**

    Note: See "Safety Information" in the *BioSprint DNA Blood Handbook*.

26. **Switch off the BioSprint 15 at the power switch.**

27. **Wipe the surface of the tube strip tray and adjacent surfaces with a soft cloth or tissue moistened with distilled water or a mild detergent solution. If infectious agents are spilt onto the tube strip tray, clean using 70% ethanol or other disinfectant.**

    Note: Do not use bleach as disinfectant. See "Safety Information" in the *BioSprint DNA Blood Handbook*.

The BioSprint 15 workstation and BioSprint 15 DNA Blood Kit are intended for life science research applications. No claim or representation is intended for their use to identify any specific organism or for a specific clinical use (diagnostic, prognostic, therapeutic, or blood banking). It is the user's responsibility to validate the performance of the BioSprint 15 workstation and BioSprint 15 DNA Blood Kit for any particular use, since their performance characteristics have not been validated for any specific organism. Purchase of products containing HotStarTaq *Plus* DNA Polymerase is accompanied by a limited license to use them in the polymerase chain reaction (PCR) process for research and development activities in conjunction with a thermal cycler whose use in the automated performance of the PCR process is covered by the up-front license fee, either by payment to Applied Biosystems or as purchased, i.e. an authorized thermal cycler. The PCR process is covered by the foreign counterparts of U.S. Patents Nos. 4,683,202 and 4,683,195 owned by F. Hoffmann-La Roche Ltd.
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Selected kit handbooks can be downloaded from **www.qiagen.com/literature/handbooks/default.aspx** .
Material safety data sheets (MSDS) for any QIAGEN product can be downloaded from **www.qiagen.com/ts/msds.asp** .

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