September 2021

Supplementary Protocol for User Self-Validation

QIAamp® DSP DNA Mini Kit Protocol for DNA Purification From Dried Blood Spots

This protocol is for purification of total (genomic, mitochondrial, and viral) DNA from blood, both untreated and treated with anticoagulants, which has been spotted and dried on filter paper (Schleicher & Schuell, no. 903), using the QIAamp DSP DNA Mini Kit (cat. no. 61304).

Note: It is the user's responsibility to validate performance using this combination for any procedures used in their laboratory.

Equipment and reagents to be supplied by user

- Ethanol (96–100%)
- Pipettes and pipette tips with aerosol barrier
- Microcentrifuge (with rotor for 2 ml tubes)
- Vortexer
- Water bath or heating block at 56°C, 70°C, and 85°C
- 3 mm single-hole paper puncher
- For the automated procedure only (QIAcube or QIAcube Connect MDx only):
  - Rotor Adapters, cat. no. 990394
  - Rotor Adapter Holder, cat. no. 990392
  - Sample Tubes CB, cat. no. 990382 (sample input tube)
  - Shaker Rack Plugs, cat. no. 9017854
  - Reagent Bottles, 30 ml, cat. no. 990393
  - Filter Tips, 1000 µl, cat. no. 990352
  - Filter Tips, 200 µl, cat. no. 990332
  - SafeSeal reaction tube, 1.5 ml, Sarstedt® (cat. no. 72.706)

Warnings and precautions

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

CAUTION: DO NOT add bleach or acidic solutions directly to the sample-preparation waste.

Lysis Buffer (AL) and Wash Buffer 1 (AW1) contain guanidine hydrochloride, which can form highly reactive compounds when combined with bleach. If liquid containing these buffers is spilt, clean with suitable laboratory detergent and water. If the spilt liquid contains potentially infectious agents, clean the affected area first with laboratory detergent and water, and then with 1% (v/v) sodium hypochlorite.

If the buffer bottles are damaged or leaking, wear gloves and protective goggles when discarding the bottles in order to avoid personal injury or injury to others.

QIAGEN has not tested the liquid waste generated by the QIAamp DSP DNA Mini procedures for residual infectious materials. Contamination of the liquid waste with residual infectious materials is unlikely, but cannot be excluded completely. Therefore, liquid waste must be considered infectious and be handled and discarded according to local safety regulations.

The following hazard and precautionary statements apply to components of the QIAamp DSP DNA Mini Kit.

Buffer AL

Contains: guanidine hydrochloride; maleic acid. Warning! May be harmful if swallowed or if inhaled. Causes skin irritation. Causes serious eye irritation. May cause an allergic skin reaction. If eye irritation persists: Get medical advice/attention. Take off contaminated clothing and wash it before reuse. Wear protective gloves/protective clothing/eye protection/face protection.

Buffer ATL

Warning! Causes mild skin irritation. If skin irritation occurs: Get medical advice/attention.

Buffer AW1

Contains: guanidine hydrochloride. Warning! Harmful if swallowed or if inhaled. Causes skin irritation. Causes serious eye irritation. Call a POISON CENTER or doctor/physician if you feel unwell. Dispose of contents/container to an approved waste disposal plant. Take off contaminated clothing and wash it before reuse. Wear protective gloves/protective clothing/eye protection/face protection.

Proteinase K

Contains: Proteinase K. Danger! Causes mild skin irritation. May cause allergy or asthma symptoms or breathing difficulties if inhaled. Avoid breathing dust/fume/gas/mist/vapors/spray. Dispose of contents/ container to an approved waste disposal plant. If experiencing respiratory symptoms: Call a POISON CENTER or doctor/ physician. IF INHALED: If breathing is difficult, remove victim to fresh air and keep at rest in a position comfortable for breathing. Wear respiratory protection.

Reagent storage and handling

QIAamp Mini spin columns should be stored at 2–8°C upon arrival. When stored properly, the QIAamp Mini spin columns are stable until the expiration date on the kit box. All buffers and Proteinase K can be stored at room temperature (15–25°C) and are stable until the expiration date on the kit box.

Reconstituted Wash Buffer 1 (AW1) and reconstituted Wash Buffer 2 (AW2) are stable at room temperature until the expiration date on the kit box.

Preparing reagents and buffers

Buffer AW1* (store at room temperature)

Buffer AW1 is supplied as a concentrate. Before using for the first time, add the appropriate amount of ethanol (96–100%) as indicated on the bottle. Reconstituted Buffer AW1 is stable at room temperature until the kit expiration date.

> **Note**: Always mix the reconstituted Wash Buffer 1 (AW1) by inverting the bottle several times before starting the procedure.

Buffer AW2† (store at room temperature)

Buffer AW2 is supplied as a concentrate. Before using for the first time, add the appropriate amount of ethanol (96–100%) to Buffer AW2 concentrate as indicated on the bottle. Reconstituted Buffer AW2 is stable for 1 year when stored closed at room temperature, but only until the kit expiration date.

> **Note**: Always mix the reconstituted Wash Buffer 2 (AW2) by inverting the bottle several times before starting the procedure.

Handling of QIAamp Mini columns

Due to the sensitivity of nucleic acid amplification technologies, the following precautions are necessary when handling QIAamp Mini columns to avoid cross contamination between sample preparations:

- Carefully apply the sample or solution to the QIAamp Mini column. Pipet the sample into the QIAamp Mini column without wetting the rim of the column.
- Always change pipette tips between liquid transfers. We recommend the use of aerosol-barrier pipette tips.
- Avoid touching the QIAamp Mini column membrane with the pipette tip.
- After all pulse-vortexing steps, briefly centrifuge the microcentrifuge tubes to remove drops from the inside of the lids.
- Open only one QIAamp Mini column at a time, and take care to avoid generating aerosols.

Wear gloves throughout the entire procedure. In case of contact between gloves and sample, change gloves immediately.

* Contains chaotropic salt. Take appropriate laboratory safety measures, and wear gloves when handling. Not compatible with disinfecting agents that contain bleach. See Warnings and precautions.

† Contains sodium azide as a preservative.

Centrifugation

All centrifugation steps should be carried out at room temperature.

Centrifugation of QIAamp Mini columns is performed at approximately 6000 x *g* to reduce centrifuge noise. Centrifugation at full speed will not affect DNA yield. Centrifugation at lower speeds is also acceptable, provided that nearly all of each solution is transferred through the QIAamp membrane.

When preparing DNA from buffy coat or lymphocytes, full-speed centrifugation is recommended to avoid clogging.

Processing QIAamp Mini columns on the QIAcube

Sample preparation using the QIAcube follows the same steps as the manual procedure (i.e., lyse, bind, wash, and elute). For more information about the automated procedure, see the relevant protocol sheet available at **www.qiagen.com/qiacubeprotocols**.

Copurification of RNA

QIAamp Mini spin columns copurify DNA and RNA when both are present in the sample. If RNA-free genomic DNA is required, 4 µl of an RNase A stock solution (100 mg/ml) should be added to the sample prior to the addition of Buffer AL. RNase A is not supplied with the kits and should be purchased separately (see Equipment and reagents to be supplied by user, page 1). Ensure that the RNase A used is free of DNase activity.

Processing QIAamp Mini columns using a microcentrifuge (spin protocols)

Close the QIAamp Mini column before placing it in the microcentrifuge. Centrifuge as described above.

- Remove the QIAamp Mini column and collection tube from the microcentrifuge. Place the QIAamp Mini column in a new collection tube. Discard the filtrate and the collection tube. Note that the filtrate may contain hazardous waste and should be disposed of appropriately.
- Open only one QIAamp Mini column at a time, and take care to avoid generating aerosols.
- For efficient parallel processing of multiple samples, fill a rack with collection tubes to which the QIAamp Mini columns can be transferred after centrifugation. Used collection tubes containing the filtrate can be discarded, and the new collection tubes containing the QIAamp Mini columns can be placed directly in the microcentrifuge.

Important points before starting

- After receiving the kit, check the kit components for damage. If the blister packs or the buffer bottles are damaged, contact QIAGEN Technical Services or your local distributor. In case of liquid spillage, refer to "Warnings and precautions", page 2. Do not use damaged kit components, since their use may lead to poor kit performance.
- Always use RNase-free equipment.
- Always change pipette tips between liquid transfers. To minimize cross-contamination, we recommend the use of aerosol-barrier pipette tips.
- All centrifugation steps are carried out at room temperature.
- Always use disposable gloves and regularly check that they are not contaminated with sample material. Discard gloves if they become contaminated.
- To minimize cross-contamination, open only one tube at a time.
- Do not use kit components from other kits with the kits you are currently using, unless the lot numbers are identical.
- Avoid microbial contamination of the kit reagents.
- To ensure safety from potentially infectious material, we recommend working under laminar airflow conditions until the samples are lysed.
- For the automation workflow, follow the instructions in the protocol sheet (QIAcube) or on the touchscreen (QIAcube Connect MDx).
- This kit should only be used by personnel trained in in vitro diagnostic laboratory practice.
- **Important note**: During incubation and water bath, temperature tolerance ranges need to be determined and validated for the respective application by the user.

Things to do before starting

- Prepare an 85°C water bath for use in step 2, a 56°C water bath for use in step 3, and a 70°C water bath for use in step 4.
- Equilibrate Elution Buffer (AE) or distilled water to room temperature for elution in step 10.
- Ensure that Buffer AW1 and Buffer AW2 have been prepared according to the instructions on page 4.
- If a precipitate has formed in Buffer AL, dissolve by incubating at 56°C.

Procedure

1. Place 3 punched-out circles from a dried blood spot into the 1.5 ml lysis tube and add 180 µl of Buffer ATL.

   Cut 3 mm (1/8 inch) diameter punches from a dried blood spot with a single-hole paper puncher.

2. Incubate at 85°C for 10 min ± 1 min. Briefly centrifuge to remove drops from inside the lid.
3. Add 20 µl proteinase K stock solution. Mix by vortexing, and incubate at 56°C for 1 h ± 10 min. Briefly centrifuge to remove drops from inside the lid.
4. Add 200 µl Buffer AL to the sample. Mix thoroughly by vortexing and incubate at 70°C for 10 min ±1 min. Briefly centrifuge to remove drops from inside the lid.

   In order to ensure efficient lysis, it is essential that the sample and Buffer AL are mixed thoroughly to yield a homogeneous solution.

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

   A white precipitate may form when Buffer AL is added to the sample. In most cases, the precipitate will dissolve during incubation. The precipitate does not interfere with the QIAamp procedure or with any subsequent application.

   Note: The following steps (steps 5–10) can be automated on the QIAcube® or QIAcube Connect MDx (cat. no. 9003070).

5. Add 200 µl ethanol (96–100%) to the sample, and mix thoroughly by vortexing. Briefly centrifuge to remove drops from inside the lid.

   It is essential that the sample and ethanol are mixed thoroughly.

6. Carefully apply the mixture from step 5 to the QIAamp Mini spin column (in a 2 ml wash tube) without wetting the rim. Close the cap, and centrifuge at approximately 6000 x *g* for ≥1 min. Place the QIAamp Mini spin column in a clean 2 ml wash tube (provided), and discard the tube containing the filtrate.

   Close each QIAamp Mini spin column in order to avoid aerosol formation during centrifugation.

7. Carefully open the QIAamp Mini spin column and add 500 µl Buffer AW1 without wetting the rim. Close the cap and centrifuge at approximately 6000 x *g* for ≥1 min. Place the QIAamp Mini spin column in a clean 2 ml wash tube (provided), and discard the tube containing the filtrate. *
8. Carefully open the QIAamp Mini spin column and add 500 µl Buffer AW2 without wetting the rim. Close the cap and centrifuge at full speed (approximately 20,000 x *g*) for 3 min ± 30 s.

* Flow-through contains Buffer AL or Buffer AW1 and is therefore not compatible with bleach. See Warnings and precautions.

9. **Recommended**: Place the QIAamp Mini spin column in a new 2 ml wash tube and discard the old tube with the filtrate. Centrifuge at full speed for ≥1 min.

   This step helps to minimize the chance of possible Buffer AW2 carryover.

10. Place the QIAamp Mini spin column in a clean Elution Tube (ET) (provided). Discard the collection tube containing the filtrate. Carefully open the QIAamp Mini spin column and add 150 µl Elution Buffer (AE). Incubate at room temperature for ≥1 min, and then centrifuge at approximately 6000 x *g* for ≥1 min.

    Important note: For all automated procedures, remove the eluates from the instrument after the finished run and store them properly.

Document Revision History

Date	Changes
09/2021	Initial release

For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN kit handbook (*QIAamp DSP DNA Mini Kit Instructions for Use*). QIAGEN kit handbooks and user manuals are available at **www.qiagen.com** or can be requested from QIAGEN Technical Services or your local distributor.

Ordering **www.qiagen.com/shop** | Technical Support **support.qiagen.com** | Website **www.qiagen.com**