QIAamp® 96 DNA QIAcube® HT Kit, Part 1

Store QIAamp 96 plates and buffers at room temperature (15–25°C). QIAGEN® Proteinase K can be stored at room temperature; to store for extended periods of time, or if the ambient temperature often exceeds 25°C, store at 2–8°C.

Further information

- *QIAamp 96 DNA QIAcube HT Handbook*: www.qiagen.com/handbooks
- Safety Data Sheets: www.qiagen.com/safety
- Technical assistance: toll-free 00800-22-44-6000, or www.qiagen.com/contact

Notes before starting

- This protocol is for purification of gDNA from blood, cells, and soft tissues (max. 20 mg), such as biopsies, mouse tails, brain, liver, and muscle using the QIAamp 96 DNA QIAcube HT Kit on the QIAcube HT and the QIAxtractor®.
- For tissue samples, please order Collection Microtube Racks (cat. no. 19560) and Collection Microtube Caps (cat. no 19566) from QIAGEN.
- Do not overload the QIAamp membrane as this can lead to impaired gDNA extraction and/or performance in downstream assays. See the kit handbook for more information on handling various sample types and instructions on how to prepare Buffers ACB, AW1, and AW2. Avoid repeated freezing and thawing of samples as this may reduce gDNA yield and quality.
- Ensure that the relevant version of the QIAamp 96 DNA *QIAcube HT*.QSP run file and software version 4.17.1 or higher are installed. This is mandatory to process the QIAamp 96 DNA QIAcube HT Kit.

gDNA purification — tissue samples

1. Cut up to 20 mg tissue (up to 10 mg spleen) into small pieces.
2. Prepare a proteinase K – Buffer ATL working solution containing 20 µl proteinase K stock solution and 180 µl Buffer ATL per sample, and mix by vortexing.

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3. Immediately pipet 200 µl working solution into each collection microtube containing the tissue samples. Seal microtubes properly with the collection microtube caps.
4. Mix the collection microtube rack by inverting with its clear cover on.
5. To collect any solution from the caps, centrifuge collection microtubes at 3000 rpm. It is essential that the samples are completely submerged in the proteinase K – Buffer ATL working solution after centrifugation.
6. Incubate shaking at 56°C overnight or until the samples are completely lysed. Place a weight on top of the caps during the incubation.
7. Shake the racks vigorously up and down for 15 s. To collect any solution from the caps, centrifuge collection microtubes at 3000 rpm.

   Optional: If RNA-free genomic DNA is required, add 4 µl RNase A (100 mg/ml).
8. Close the collection microtubes with fresh caps, mix by shaking vigorously, and incubate for 5 min at room temperature. To collect any solution from the caps, centrifuge collection microtubes at 3000 rpm.
9. Carefully remove caps and transfer the lysate into a new S-Block and proceed with step 1 of part 2 of the protocol.

gDNA purification — blood and cell samples

1. Add 200 µl samples to S-Block wells.

   Note: If working with a sample volume of <200 µl, adjust the volume to 200 µl with PBS buffer. If working with cultured cells, centrifuge the appropriate number if cells (max. 5 x 10^6) for 5 min at 300 x *g*. Resuspend the pellets in 200 µl PBS buffer each.

   Optional: If RNA-free genomic DNA is required, add 4 µl RNase A (100 mg/ml) and incubate for 2 min at room temperature.
2. Continue with step 1 in part 2 of the protocol.