User-developed protocol

User-Developed Protocol:

Purification of total DNA from animal sperm using the DNeasy® Blood & Tissue Kit; protocol 1

This procedure has been adapted by customers from the DNeasy tissue protocol and is for purification of DNA from fresh or frozen animal semen samples using the DNeasy Blood & Tissue Kit. **It has not been thoroughly tested and optimized by QIAGEN.**

IMPORTANT: Please read the "Safety Information" and "Important Notes" sections in the *DNeasy Blood & Tissue Handbook* before beginning this procedure. For safety information on the additional chemicals mentioned in this protocol, please consult the appropriate material safety data sheets (MSDSs), available from the product supplier. DNeasy Blood & Tissue Kits are intended for research use. No claim or representation is intended to provide information for the diagnosis, prevention, or treatment of a disease.

Equipment and reagents to be supplied by user

- DNeasy Blood & Tissue Kit (cat. no. 69504 or 69506)
- Pipets and pipet tips
- Corex® centrifuge tubes*
- Vortexer
- Microcentrifuge tubes (2 ml)
- Microcentrifuge with rotor for 1.5 ml and 2 ml tubes
- Thermomixer, shaking water bath, or rocking platform for heating at 56°C
- Ethanol (96–100%)†
- Additional proteinase K (cat. no. 19131 or 19133) and Buffer AL (cat. no. 19075) may be needed if the DNeasy Blood & Tissue Kit is used mainly for sperm samples
- Buffer 1:
  150 mM NaCl
  10 mM EDTA, pH 8.0
- Buffer 2:
  100 mM Tris·Cl, pH 8.0
  10 mM EDTA
  500 mM NaCl
  1% SDS
  2% β-mercaptoethanol

* This is not a complete list of suppliers and does not include many important vendors of biological supplies. However, use of Corex centrifuge tubes is strongly recommended since sperm cells do not adhere strongly to them. Use of other tubes may lead to significantly reduced yields.

† Do not use denatured alcohol, which contains other substances such as methanol or methylethylketone.

Important points before starting

- If using the DNeasy Blood & Tissue Kit for the first time, read “Important Notes” in the *DNeasy Blood & Tissue Handbook*.
- All centrifugation steps are carried out at room temperature (15–25°C) in a microcentrifuge.
- Vortexing should be performed by pulse-vortexing for 5–10 s.

Things to do before starting

- Prepare Buffer 1 and Buffer 2, as described above.
- Buffer ATL and Buffer AL may form precipitates upon storage. If necessary, warm to 56°C until the precipitates have fully dissolved.
- Buffer AW1 and Buffer AW2 are supplied as concentrates. Before using for the first time, add the appropriate amount of ethanol (96–100%) as indicated on the bottle to obtain a working solution.
- Preheat a thermomixer, shaking water bath, or rocking platform to 56°C for use in step 9.

Procedure

1. **Add 50–250 µl sperm to 10 ml Buffer 1 in a Corex centrifuge tube. Vortex for 10 s at full speed.**

   Note: Corex centrifuge tubes are strongly recommended since sperm cells do not adhere strongly to these.
2. **Centrifuge for 10 min at 4000 rpm (2500 x *g*).**
3. **Carefully remove the supernatant, leaving approximately 1 ml of pellet and Buffer 1.**
4. **Vortex for 10 s at full speed and transfer into a 2 ml microcentrifuge tube.**
5. **Add 0.5 ml Buffer 1 to the Corex tube and vortex for 10 s at full speed to collect any sample adhering to the walls of the tube. Transfer into the same microcentrifuge tube.**
6. **Centrifuge for 2 min at full speed in a microcentrifuge.**
7. **Carefully remove the supernatant.**

   Note: Do not remove any of the semen pellet.
8. **Resuspend the pellet in 300 µl Buffer 2.**
9. **Add 100 µl proteinase K and incubate for 2 hours at 56°C. Vortex occasionally during incubation to disperse the sample, or place in a thermomixer, shaking water bath, or on a rocking platform.**
10. **Add another 20 µl proteinase K and incubate for a further 2 hours at 56°C. Vortex occasionally during incubation to disperse the sample, or place in a thermomixer, shaking water bath, or on a rocking platform.**

11. **Add 400 µl Buffer AL to the sample, and mix thoroughly by vortexing. Then add 400 µl ethanol (96–100%), and mix again thoroughly by vortexing.**

    It is essential that the sample, Buffer AL, and ethanol are mixed immediately and thoroughly by vortexing or pipetting to yield a homogeneous solution. Buffer AL and ethanol can be premixed and added together in one step to save time when processing multiple samples.

    A white precipitate may form on addition of Buffer AL and ethanol. This precipitate does not interfere with the DNeasy procedure.

12. **Pipet up to 650 µl of the mixture from step 11 (including any precipitate) into the DNeasy Mini spin column placed in a 2 ml collection tube (provided). Centrifuge at ≥6000 x *g* (8000 rpm) for 1 min. Discard flow-through and reuse the collection tube.***

13. **Repeat step 12 until all of the sample has been loaded.**

14. **Place the DNeasy Mini spin column in a new 2 ml collection tube (provided), add 500 µl Buffer AW1, and centrifuge for 1 min at ≥6000 x *g* (8000 rpm). Discard flow-through and collection tube.***

15. **Place the DNeasy Mini spin column in a new 2 ml collection tube (provided), add 500 µl Buffer AW2, and centrifuge for 3 min at 20,000 x *g* (14,000 rpm) to dry the DNeasy membrane. Discard flow-through and collection tube.**

    It is important to dry the membrane of the DNeasy Mini spin column, since residual ethanol may interfere with subsequent reactions. This centrifugation step ensures that no residual ethanol will be carried over during the following elution.

    Following the centrifugation step, remove the DNeasy Mini spin column carefully so that the column does not come into contact with the flow-through, since this will result in carryover of ethanol. If carryover of ethanol occurs, empty the collection tube, then reuse it in another centrifugation for 1 min at 20,000 x *g* (14,000 rpm).

16. **Place the DNeasy Mini spin column in a clean 1.5 ml or 2 ml microcentrifuge tube (not provided), and pipet 200 µl Buffer AE directly onto the DNeasy membrane. Incubate at room temperature for 1 min, and then centrifuge for 1 min at ≥6000 x *g* (8000 rpm) to elute.**

    Elution with 100 µl (instead of 200 µl) increases the final DNA concentration in the eluate, but also decreases the overall DNA yield (see *DNeasy Blood & Tissue Handbook*).

17. **Recommended: For maximum DNA yield, repeat elution once as described in step 16.**

    This step leads to increased overall DNA yield.

    A new microcentrifuge tube can be used for the second elution step to prevent dilution of the first eluate. Alternatively, to combine the eluates, the microcentrifuge tube from step 16 can be reused for the second elution step.

    Note: Do not elute more than 200 µl into a 1.5 ml microcentrifuge tube because the DNeasy Mini spin column will come into contact with the eluate.

* Flow-through contains Buffer AL or Buffer AW1 and is therefore not compatible with bleach. See *DNeasy Blood & Tissue Handbook* for safety information.

Troubleshooting

For general troubleshooting, please consult the Troubleshooting Guide in the *DNeasy Blood & Tissue Handbook*.

	Comments and suggestions
Low yield	
Corex centrifuge tubes not used in step 1	Use of Corex centrifuge tubes is strongly recommended since sperm cells do not adhere to them. Use of other tubes may lead to significantly reduced yields.