

User-Developed Protocol:

Isolation of genomic DNA from sperm using the QIAamp[®] DNA Mini Kit; protocol 1

This procedure has been adapted by customers from the QIAamp[®] Tissue Protocol, and is for use with the QIAamp DNA Mini Kit. **It has not been thoroughly tested and optimized by QIAGEN.**

The protocol can be used for fresh or frozen semen samples with equal efficiency. Frozen samples must be thawed thoroughly before use. Please note that lysis time will vary depending on the size and density of the source material.

Please be sure to read the QIAGEN[®] *QIAamp DNA Mini Kit and QIAamp DNA Blood Mini Kit Handbook* and detailed Tissue Protocol carefully before beginning this procedure.

Note: Proteinase K is recommended instead of QIAGEN Protease in this procedure, because QIAGEN Protease is incompatible with Buffer 2.

Procedure

1. **Add 50–250 µl sperm to 10 ml Buffer 1 in a Corex[®] centrifuge tube. Vortex for 10 s at full speed.**
Buffer 1: 150 mM NaCl, 10 mM EDTA (pH 8.0).
Note: Only use Corex tubes as 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 ~ 1 ml of pellet and Buffer 1.**
4. **Vortex for 10 s at full speed and transfer into a 2.0 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.**
Buffer 2:
100 mM Tris·Cl (pH 8.0)
10 mM EDTA
500 mM NaCl
1% SDS
2% β-mercaptoethanol*

* **Note:** β-mercaptoethanol is toxic; dispense in a fume hood and wear appropriate protective clothing.

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9. Add 100 µl Proteinase K solution, provided, and incubate for 2 hours at 55°C. Invert the tube occasionally to disperse the sample, or place on a rocking platform.
10. Add another 20 µl Proteinase K and incubate for a further 2 hours at 55°C. Invert the tube occasionally to disperse the sample, or place on a rocking platform.
11. Add 400 µl Buffer AL and 400 µl ethanol to the sample and mix by vortexing.
12. Continue with step 5 of the Tissue Protocol in the *QIAamp DNA Mini Kit and QIAamp DNA Blood Mini Kit Handbook*.

Note: Repeat step 5 in the Tissue Protocol to apply all the lysate to the QIAamp Spin Column.

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Trademarks: QIAGEN[®], QIAamp[®] (QIAGEN); Corex[®] (Corning, Inc.).
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