User-Developed Protocol

Rapid extraction of DNA from sperm using the EZ1® DNA Investigator® Kit

This protocol has been adapted by the North Louisiana Criminalistics Laboratory from the pretreatment for epithelial cells mixed with sperm cells and is for the lysis and extraction of DNA from a sperm pellet after differential separation. The EZ1 DNA Investigator protocol "Pretreatment for Epithelial Cells Mixed with Sperm Cells" should be followed up to the point where a clean sperm pellet is isolated.

This protocol has not been thoroughly tested and optimized by QIAGEN.

IMPORTANT: Please read the "Safety Information" and "Important Notes" sections in the *EZ1 DNA Investigator Handbook* before beginning this procedure. For safety information on the additional chemicals mentioned in this protocol, please consult the appropriate safety data sheets (SDSs), available from the product supplier.

Equipment and reagents to be supplied by user

For all users

- 1 M dithiothreitol (DTT)
- Pipet tips (pipet tips with aerosol barriers to prevent cross-contamination are recommended)
- 10–200 µl pipettor
- Microcentrifuge
- Thermomixer or orbital incubator
- Ultrasonicator bath (recommended)

For EZ1 Advanced users

- EZ1 Advanced instrument (cat. no. 9001410)
- EZ1 DNA Investigator Kit (cat. no. 952034)
- EZ1 Advanced DNA Investigator Card (cat. no. 9018302)

For EZ1 Advanced XL users

- EZ1 Advanced XL instrument (cat. no. 9001492)
- EZ1 DNA Investigator Kit (cat. no. 952034)
- EZ1 Advanced XL DNA Investigator Card (cat. no. 9018699)

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For BioRobot® EZ1 workstation users

- BioRobot EZ1 workstation
- EZ1 DNA Investigator Kit (cat. no. 952034)
- EZ1 DNA Investigator Card (cat. no. 9016387)

Important points before starting

- If using the EZ1 DNA Investigator Kit for the first time, read “Important Notes” in the *EZ1 DNA Investigator Handbook*.
- The differential wash protocol uses Buffer G2 as a wash buffer. Additional Buffer G2 (cat. no. 1014636) should be purchased separately.

Things to do before starting

- Heat a thermomixer or orbital incubator to 70°C for the proteinase K digest in step 4.

Procedure

1. **Follow the protocol “Pretreatment for Epithelial Cells Mixed with Sperm Cells” to the point where a clean sperm pellet is isolated.**
2. **Resuspend the sperm pellet in 160 µl Buffer G2.**
3. **Add 10 µl proteinase K and 40 µl 1 M DTT. Vortex to mix.**
4. **Incubate at 70°C for 10 min in a thermomixer or orbital incubator at 850 rpm.**
5. **For maximum recovery, place samples in an Ultrasonicator for 10 min. Alternatively, vortex vigorously for 10 s.**

   Note: A 2 ml EZ1 DNA Investigator sample tube should be used.
6. **Continue with the protocol “DNA Purification (Trace Protocol)”.**