DNA: Differential Extraction

Sample types

QAS 9.1.1.b

Prior to the organic or robotic extraction of sexual assault samples containing sperm, both the epithelial and sperm cells must be prepared.

Chemicals, reagents, equipment and supplies

Refer to *DNA: Organic Extraction* and *SER: Spermatozoa – Microscopic Examination* for a list of chemicals, reagents, equipment, and supplies used in these procedures.

Differential lysis

The following procedure is used to differentially lyse the cells. The volumes of reagents used varies based on the extraction method.

NOTE: If samples containing sperm are going directly to Y-STR analysis only, proceed to *Sperm cell lysis*.

Step	Action
1	To the approximately 30 µL of re-suspended cell pellet and/or substrate, and the extraction blanks, add Digest Buffer: • robotic extraction: 200 – 300 µL • organic extraction: 500 µL
2	Add 15 µL of Proteinase K Solution. Mix gently.
3	Incubate at 56° ± 1°C for at least one hour to lyse the epithelial cells. **Do not** incubate for more than two hours to minimize lysis of sperm. Remove the substrate, if present. Place the substrate in an autoclaved spin basket and return it to the sample tube prior to centrifuging. NOTE: In cases where the sample has been consumed, retain the substrate.
4	Centrifuge the sample for five minutes on high speed and at room temperature. Remove the spin basket, if present.
5	Transfer the supernatant to a new sterile microcentrifuge tube or EZ1 sample tube. This epithelial cell material will be set aside and may be extracted in parallel with the lysed sperm fraction.

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DNA: Differential Extraction, Continued

Differential lysis (continued)

Step	Action
6	For sexual assault scenarios with no consenting partner and only one assailant, further analysis of the epithelial cell fraction is not required and the non-sperm fraction may be retained. For male sexual assault scenarios where the cell type of interest is nucleated epithelial cells (contact/saliva), sperm cell lysis is not required and the sperm fraction may be retained.
7	Wash the sperm cell pellet by suspending the pellet in Digest Buffer and vortexing briefly: • Robotic extraction: 200-300 µL • Organic extraction: 500 µL
8	Centrifuge the sample for five minutes on high speed and at room temperature.
9	Remove and discard all but approximately 50 µL of the supernatant.
10	Repeat Steps 7-9 an additional two to four times to remove residual epithelial cell material. Proceed to *Sperm cell lysis*. NOTE: It is optional to make a slide at this time to verify the presence of spermatozoa. Proceed to *Pellet examination* if making a slide.

Pellet examination

If making a slide, use the following procedure to examine the pellet.

Step	Action
1	Re-suspend the pellet in sterile water and vortex briefly: • Robotic extraction: 200-300 µL • Organic extraction: 500 µL
2	Centrifuge the sample for five minutes on high speed and at room temperature.
3	Remove and discard all but approximately 50 µL of the supernatant.
4	Re-suspend the sperm pellet in the remaining approximately 50 µL of sterile water by stirring it with a sterile pipette tip.
5	Dry 2 µL of re-suspended sample on a microscope slide for a minimum of 10 minutes in a 60° oven or on a heat block.

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DNA: Differential Extraction, Continued

Pellet examination (continued)

Step	Action
6	Stain with *Christmas Tree Stain A* for 5 to 30 minutes. Rinse with deionized water.
7	Stain with *Christmas Tree Stain B* for 5 to 60 seconds. Rinse with ethanol.
8	Allow the slide to dry. Add mounting medium and a coverslip.
9	Grade the sperm observed and record the results. Proceed to *Sperm cell lysis*.

Sperm cell lysis

Use the following procedure to lyse the sperm cells. The volumes of reagents used varies based on the extraction method.

Step	Action
1	Add Digest Buffer to the sperm cell pellet or Y-STR substrate: • robotic: 150 µL • Y-STR substrate and robotic extraction: 200 µL • organic: 500 µL
2	Add 20 µL of 1 M Dithiothreitol.
3	Add 15 µL of Proteinase K Solution.
4	Incubate the sample at 56° ± 1°C for at least 60 minutes. If the Y-STR substrate is present, place it in an autoclaved spin basket, return the spin basket to the sample tube, and centrifuge the sample for five minutes at high speed and room temperature. NOTE: If the sample has been consumed, retain the substrate.
5	For organic extraction samples **only**: add 15 µL of Proteinase K to each sperm and reserved epithelial cell fraction. Incubate at 56° ± 1°C overnight.

DNA extraction

For samples that will be extracted using the robot, proceed to *DNA: EZ1 Advanced XL Extraction, Set-up and operation of the BioRobot EZ1*.

For samples that will be extracted using the organic method, proceed to *DNA: Organic Extraction, DNA extraction and DNA concentration and wash (Vivacon)*.