Sampletype **i-sep**® DL – Workflow of an Immunological Test and a Subsequent Differential Lysis Using the Identical Sample

Abstract

DNA from epithelial cells can be removed using a procedure called a differential lysis, which takes advantage of unique properties associated with each cell type. The cells are removed from the suspected material by soaking them in a gentle solution. Epithelial cell DNA (such as those found in vaginal fluid) is isolated under mild conditions that break open the epithelial cells but leave the sperm cells DNA intact. The DNA in spermatozoa can then be extracted using a more harsh extraction procedure. For a successful experiment, please ensure that the DNA of epithelial cells is completely removed from the sample. This means existing protocols might be optimized to the Sampletype **i-sep**® DL technology to guarantee best results.

- Make sure all epithelial cells are lysed during the first lysis
- Before carrying on with the second lysis, please ensure all DNA from epithelial cells is removed properly.
- Use an additional washing step if needed

Equipment and Reagents to Be Supplied by User

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, consult the appropriate material safety data sheets (MSDSs), available from the product supplier.
The system is very flexible in terms of the applied lysis conditions and the required reagents.

- Lysis buffer
- Proteinase K

- Pipets (adjustable)
- Sterile pipet tips (pipet tips with aerosol barriers are recommended to help prevent cross-contamination)
- Incubator or alternatively a heating block or similar at 56°C (capable of holding 2 ml collection tubes)
- Microcentrifuge

Preparation of lysis buffer 1 and 2

Mix 970 µl buffer G2[1] per sample + 30 µl proteinase K and split the solution in two parts

- 500 µl are used as **Lysis buffer 1**
- For **Lysis buffer 2** add additionally 20 µl 1 M DTT to the other 500 µl of the mixture

[1] Lysis buffer for isolation of genomic DNA - QIAGEN (Cat. No. 1014636)

Immunological test for semen

- Place the filter column in a 2 ml collection tube
- Place the sample in the filter column (approx. **5 x 5 mm**)
- Add 100 µl Extraction buffer of the immunological test system (e.g. RSID Semen, Galantos GmbH)
- Incubate the assembly at the bench (room temperature) for **60 minutes**
- Centrifuge the Sampletype assembly at 7,000 rpm for 2 min
- Extract can be used in the immunological test according to the manufacturer's instructions

Lysis of epithelial cells (1. Lysis step)

- Filter column stays in the 2 ml collection tube
- Add 500 µl **0.25% acetic acid** (~56°C), close the lid
- Incubate at 56°C for **30 min** (no need to be shaken)
- Centrifuge the Sampletype at 7,000 rpm for 2 min
- Please check if all liqid was removed; otherwise repeat the centrifugation step
- Place the filter column in a fresh 2 ml collection tube
- Add 500 µl **Lysis buffer 1** (~56°C), close the lid
- Incubate the assembly at 56°C for **105 min** (no need to be shaken)
- Centrifuge the Sampletype assembly at 7,000 rpm for 2 min
- Place the filter column in a fresh collection tube
- Close the lid of the collection tube and store the lysate for further processing; tube contains 500 µl **Lysat 1** containing the DNA isolated from the epithelial cells for further purification

Lysis of sperm (2. Lysis step)

- Add 500 µl **Lysis buffer 2** to the sample into the filter column placed in a fresh collection tube
- Incubate **over night** at 56°C (no need to be shaken)
- Centrifuge the Sampletype assembly at 7,000 rpm for 2 min
- Remove the filter column
- Close the lid of the collection tube and store the lysate for further processing; tube contains 500 µl **Lysat 2** containing the DNA isolated from the sperm cells for further purification

NOTE! For subsequent DNA-purification on an automated platform place the 2 ml collection tube on the rack. Cut-off the lid and store the filter unit for further investigations.