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SOP – CSF cfDNA sequencing

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This standard operating procedure describes the procedure for the sampling and initial preparation of CSF and blood for cell-free DNA (cfDNA) sequencing.

For any questions, issues or remarks, please reach out to as at the following email-address:

liquidbiopsy.NEU@med.uni-heidelberg.de

Thank you!

The liquid biopsy team, Universitätsklinikum Heidelberg

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Materials:

Material	Catalogue number
Centrifuge for 15 ml tubes (1.800xg)	
Centrifuge for 2 ml tubes (15.000xg)	
Falcon 15 mL	Falcon™ 352095
Eppendorf LoBind-Tube 2 mL	Eppendorf catalogue nr.: 0030108078
Eppendorf LoBind-Tube 1.5 mL	Eppendorf catalogue nr.: 0030108051
Pasteur pipets	
P1000 Pipet with filtered tips	

Core principles:

- **A quick preparation of the samples is essential.**

  The cells contained (in different proportions) in diverse body fluids undergo lysis after sampling and release their DNA in the fluid. This cell/genomic DNA pollutes and dilutes cell-free DNA (cfDNA) and is very difficult or even impossible to distinguish from it. This leads to a reduced proportion of cfDNA and distorts the sequencing results. Therefore, a quick preparation is essential not only in cell-rich fluids (blood, …), but also in cell-arm fluids (CSF, …). Additionally, with time, DNA is degraded (by the nucleases of the fluid), which also distorts results.

- **Prolonged storage should be done in special cfDNA tubes (Streck / Roche™).**

  Should the immediate sample preparation not be possible (late afternoon, weekend), the samples must be stored in special cfDNA tubes (Streck cell-free BCT) at room temperature (blood in a refrigerator, if not in a Streck tube). This reduces cell lysis as well as cfDNA degradation.

  However, the supernatant should ideally be separated from the cell pellet as soon as possible to prevent the contamination of the sample with leucocyte genomic DNA. After centrifugation, the supernatant may be stored in a classical tube (Falcon) or 1,5 mL Eppendorf LoBind tube.

Procedure:

Remark: All samples should be identified at all times with patient data and nature of the sample.

Step 1: storage of EDTA-blood

1. Invert the EDTA tube 10 times for a good homogenisation.
2. **Open the EDTA tube and transfer its contents into a Falcon tube (15 mL).**
3. Freeze the whole blood at -80 °C.

Step 2: Separating the sample into its constituents

1. Cool down the centrifuges to 22°C.
2. **First centrifugation** of the CSF in a Streck Cell-Free DNA tube (or Falcon if cfDNA tube not available) at 1.800xg for 10 min at 22°C.
3. Transfer the supernatant with a Pasteur pipet into separate 2 mL Eppendorf LoBind tubes (for a 5 mL sample, 2x 2mL and 1x1 mL).

*Be careful not to disturb the cell pellet, usually not or only slightly visible. In this step, less than 500 µL should be lost with the cell pellet.

4. **Second centrifugation** of the supernatant extracted in step 3 in 2 mL Eppendorf LoBind-Tubes at 16.000xg for 10 min at 22°C.
5. Transfer the supernatant after the 2. centrifugation from all the 2 mL Eppendorf tubes with a P1000-Pipette into 1-2 15 mL Falcon tubes. Under no circumstance should the cell pellet at the bottom of the Eppendorf tubes be transferred with the supernatant. It is usually not visible.

*The first Falcon should contain max. 5 mL CSF supernatant (label CSF1). Excess supernatant should be stored in a second tube (label CSF backup).

Step 3: Storage of the samples until dispatching

CSF and whole blood:

- Short-term storage (1 day): -20°C
  - Due to transportation time, samples should be frozen at -80°C before dispatching
- Long-term storage: -80°C

Step 4: Dispatching of the samples and registration

Send the samples and following documents to the “Liquid Biopsy Lab Hirntumore” of the university hospital Heidelberg:

Samples: (must be on dry ice if no Streck tube used, preferably on ice if Streck tube used for sampling)

- CSF supernatant (without cells)
- EDTA-blood in Falcon

Documents:

- Filled-out request form

Destination:

Universitätsklinik Heidelberg, Kopfklinik
Liquid Biopsy Lab Hirntumore
Im Neuenheimer Feld 400
69120 Heidelberg
Phone number: +49 6221 56-35603 (Sandy Walter) or +49 6221 56-38265 (Andrea Dormann)

After dispatch, please report the dispatch (WITHOUT patient name) to our central email address: liquidbiopsy.NEU@med.uni-heidelberg.de
You will be informed of the arrival and analysis of your samples over this email address.