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Liquid Biopsy Diagnostics from CSF – SOP for initial sample processing

For in-house CSF laboratories

External referrers (International)

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Purpose of the Document

This SOP describes the preparation of CSF samples in external centers for shipment for liquid biopsy diagnostics of brain tumors.

For questions, please contact:
liquidbiopsy.NEU@med.uni-heidelberg.de

Thank you for your support.

Liquid Biopsy Lab Brain Tumors
Heidelberg University Hospital

Authors of this Edition

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Materials

Material	Catalog Number	Storage Location
Centrifuge for 15 mL tubes (1,800 × g)		
Centrifuge for 2 mL tubes (16,000 × g)		
Falcon 15 mL	Falcon™ 352095	
Eppendorf LoBind tube 2 mL	Eppendorf Cat. No.: 0030108078	
Eppendorf LoBind tube 1.5 mL	Eppendorf Cat. No.: 0030108051	
Sterile serological pipettes (2.5 mL)		
P1000 pipette with filter tips		

Important Principles:

Rapid Sample Processing

Timely processing of samples is crucial. After collection, cellular components may degrade and release genomic DNA, which dilutes or contaminates cell-free DNA (cfDNA). This can significantly impair sequencing quality.

Even in low-cellularity samples such as CSF, DNA degradation due to nucleases may occur.

Storage in Cell-free DNA Tubes

If immediate processing is not possible, samples should be stored at room temperature in dedicated cfDNA tubes (e.g., Streck, Roche, PAXgene, etc.).

Blood samples without cfDNA tubes should be stored refrigerated.

However, early separation of cell pellet and supernatant is optimal.

Shipping Conditions

If CSF was collected in a cell-free DNA tube (Streck, Roche, etc.):
→ **Ship at room temperature**

If CSF was NOT collected in a cfDNA tube:
→ **Ship on dry ice**

The blood sample may be shipped either at room temperature or on dry ice regardless of tube type.

Protocol

Note: All samples must be clearly labeled with patient identifiers.

Step 1: EDTA Whole Blood

1. Gently invert the EDTA tube 10 times.

2. Gently unscrew EDTA tube and transfer contents into a 15 mL Falcon tube.

3. Store at -80 °C until shipment.

Step 2: Centrifugation of CSF Sample

1. **Preparation**
   Pre-cool centrifuges:
   - CSF in cfDNA tubes → 22 °C
   - CSF in standard tubes → 4 °C

2. **First centrifugation**
   1.800 × g for 10 minutes
   - CSF in cfDNA tubes → 22 °C
   - CSF in standard tubes → 4 °C

3. **Transfer of supernatant**:
   Carefully transfer supernatant using a serological pipette into 2 mL Eppendorf LoBind tube.

   Note: Ensure that the cell pellet is not disturbed (usually not visible). Normally, no more than 500 µL CSF should be lost.

4. **Second centrifugation:** (supernatants from step 2.3.)
   16,000 × g for 10 minutes
   - CSF in cfDNA tubes → 22 °C
   - CSF in standard tubes → 4 °C

5. **Pooling of supernatant**:
   Transfer supernatant using a P1000 pipette into Falcon tubes:

   - **Tube 1**: max. 5 mL supernatant (Label: CSF Sample)
   - **Tube 2**: remaining supernatant (Label: CSF Backup)

   Note: Ensure that the pellet in the 2 mL LoBind tubes is not disturbed.

Step 3: Sample Storage Until Shipment

1. **Storage of CSF**:
    - **CSF collected in cell-free DNA tube (Streck): room temperature**
    - **CSF collected in standard tube:**
        - Short-term storage (1 day): **- 20 °C**
        - Long-term storage: **- 80 °C**

2. **Storage of EDTA Whole Blood**:
    - **Room temperature or refrigerated** (depending on CSF tube type)
        - Short-term storage: (1 day): **- 20 °C**
        - Long-term storage: **- 80 °C**

Step 4: Sample Shipment

Ship the following samples and documents to the Liquid Biopsy Lab Brain Tumors of Heidelberg University Hospital.

Samples: (observe shipping conditions depending on tube type)

- **CSF supernatant**
- **EDTA Whole Blood**

Documents:

- **Completed submission form**
- **Cost coverage document**:
    - **Submission from EU countries**:
      Issuance of an S2 form with signature and official stamp.
    - **Submission from non-EU countries**:
      Please contact the International Office of Heidelberg University Hospital to clarify cost coverage. Once cost coverage has been confirmed, the sample may be submitted.
      (Email: international.office@med.uni-heidelberg.de)

NOTE: In the absence of a valid S2 form or written confirmation of cost coverage from the International Office, samples will **NOT** be processed!

Shipping Address:

Liquid Biopsy Lab Hirntumore
Attn. Andrea Dormann und Sandy Walter
Universitätsklinik Heidelberg, Kopfklinik
Im Neuenheimer Feld 400
69120 Heidelberg
Germany

Phone: +49 6221 56-38265 (Andrea Dormann)
or +49 6221 56-35603 (Sandy Walter)

Sample Registration:

Please announce sample shipment by email (without patient identifiers):
liquidbiopsy.NEU@med.uni-heidelberg.de

You will receive a confirmation of receipt and further information on processing status via this email address.