

cfDNA extraction from peripheral blood

Equipment

Equipment	Source
Streck - Cell-Free DNA BCT® (10 mL, CE-IVD) *	https://www.streck.com/products/stabilization/cell-free-dna-bct/
BD Vacutainer EDTA Plus tubes, PET, 10 mL, K2 EDTA*	https://www.megro.de/bd-vacutainer-edta-plus-tubes-pet-10-ml-10-8-mg-k2-edta-10-x-16-ml-bec-367525.html

*Depending on protocol choice, either Streck or EDTA tubes are required

Kits

To get a complete list on equipment and reagents please relate to the manual of a corresponding kit

Kit	Source
QIAamp Circulating Nucleic Acid Kit (Qiagen)	https://www.qiagen.com/de-us/products/discovery-and-translational-research/dna-rna-purification/dna-purification/cell-free-dna/qiaamp-circulating-nucleic-acid-kit

Optional equipment

- We strongly recommend using QIAvac 24 Plus, QIAvac Connecting System, and Vacuum Pump for cfDNA extraction
- Lab Freezer capacity for plasma storage (-80°C), cfDNA storage (-20°C)

Standard Operating Procedure

1. Blood plasma collection

a. EDTA tubes

- An authorized healthcare professional collects overall 20 mL blood into two 10 mL BD Vacutainer EDTA Plus tubes
- Blood is stored at 4 °C until centrifugation
- Blood is centrifuged at 300 xg within 2-4 hours after the blood draw (to prevent cfDNA contamination through genomic DNA)
- blood plasma is collected into a new 15 mL Falcon tube

b. Streck tubes

- An authorized healthcare professional collects overall 20 mL blood into two 10 mL Streck - Cell-Free DNA tubes
- Blood sample can be stored up to 14 days at 6 °C - 37 °C
- Blood is centrifuged at 300 xg
- blood plasma is collected into a new 15 mL Falcon tube

2. Blood plasma storage (optional)

- Blood plasma can be optionally stored at -80°C for up to 2 years until further usage
- Freeze-thawing cycles should be omitted to prevent cfDNA degradation

3. cfDNA extraction

cfDNA is extracted according to the manufacturer's manual with following specifications:

- No carrier RNA is added to the AVE buffer
- Elution is performed in two steps with 10-minute incubation time each. Elution volumes are 40 This improves the cfDNA yield

4. Library preparation and DNA sequencing

Perform library preparation and sequencing on a sequencing platform of choice. cfDNA has a notoriously low yield, and should be used cautious with high input sequencing protocols.