

## **Blood Plasma Preparation for Protein and DNA Analysis**

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### **General**

- ❑ Use EDTA as an anticoagulant
- ❑ Withdraw 4 10 ml samples
- ❑ Note the time of collection
- ❑ Use one of the three basic protocols below. Note which one is used.
- ❑ Note the time of cooling, if applicable
- ❑ Forward samples to biobank
- ❑ Note the time of freezing

### **EDTA Plasma\* Protocol 1 (plasma with residual platelet release)**

- ❑ Blood samples are collected into 4 10 ml K<sub>2</sub>EDTA tubes, inverted 10 times, and stored at room temperature, until centrifuged
- ❑ The blood is centrifuged at 1300 RCF for 10 minutes at 2-6°C.
- ❑ The supernatant (plasma) is transferred to a new tube and centrifuged at 2400 RCF for 15 minutes at 2-6°C.
- ❑ (The “buffy coat” containing white cells, can be saved separately as a source of DNA)
- ❑ Samples are aliquoted into cryovials and frozen in liquid nitrogen, without delay.
- ❑ All aliquoting and freezing should be complete within 75 to 90 minutes.
- ❑ Note actual time of withdrawal and of freezing (even if target times are exceeded), so actual processing time can be calculated
- ❑ Enter data into database in accordance with legal and contractual requirements
- ❑ The plasma and “buffy coat” is stored at -70°

### **EDTA Plasma\* Protocol 2a (reduced platelet release, apparently equivalent result as EDTA Plasma Protocol 2b)**

- ❑ Blood samples are collected into 4 10 ml K<sub>2</sub>EDTA tubes, inverted 10 times, and stored at room temperature, until centrifuged
- ❑ Immediately after withdrawal, plasma is obtained by centrifugation for 10 minutes at 2000 RCF at room temperature.

- ❑ (The “buffy coat” containing white cells, can be saved separately as a source of DNA)
- ❑ The supernatant is removed and filtered through a cellulose acetate filter unit with 0.2 µm pore size and 5 cm<sup>2</sup> filtration area.
- ❑ Samples are aliquoted into cryovials and frozen in liquid nitrogen, without delay.
- ❑ All aliquoting and freezing should be complete within 75 to 90 minutes.
- ❑ Note actual time of withdrawal and of freezing (even if target times are exceeded), so actual processing time can be calculated
- ❑ Enter data into database in accordance with legal and contractual requirements
- ❑ The plasma and “buffy coat” is stored at –70°

**EDTA Plasma\* Protocol 2b (reduced platelet release, apparently equivalent result as EDTA Plasma Protocol 2a)**

- ❑ Blood samples are collected into 4 10 ml K<sub>2</sub>EDTA tubes, inverted 10 times, and stored at room temperature, until centrifuged.
- ❑ Immediately after withdrawal, plasma is obtained by centrifugation for 10 minutes at 2000 RCF at room temperature.
- ❑ (The “buffy coat” containing white cells, can be saved separately as a source of DNA)
- ❑ The supernatant is removed and subjected to centrifugation for 15 minutes at 2500 RCF at room temperature.
- ❑ Samples are aliquoted into cryovials and frozen in liquid nitrogen, without delay.
- ❑ All aliquoting and freezing should be complete within 75 to 90 minutes.
- ❑ Note actual time of withdrawal and of freezing (even if target times are exceeded), so actual processing time can be calculated
- ❑ Enter data into database in accordance with legal and contractual requirements
- ❑ The plasma and “buffy coat” is stored at –70°

\*For general-purpose proteomic screening applications, EDTA plasma is currently preferred: EDTA plasma>Citrate plasma>Serum. Serum can be used for validation studies, with qualifications. Protocol 2a and 2b have reduced release of platelet-derived proteins. Protocol 1 may have less protein degradation.