

The Education, Scholarships, Apprenticeships and Youth Entrepreneurship  
Programme – EEA Grants 2014-2021

## **#9 COLLECTION AND PROCESSING OF SAMPLES FOR PROTEOMIC STUDIES**

### **COLLECTION OF SAMPLES FOR PROTEOMIC STUDIES**

Research Center for Advanced Medicine – Medfuture (UMFIH) can receive and process various biological samples, including liquid biopsies like blood, urine and saliva samples, but also solid biopsies meaning mainly tissue samples. All samples need to be collected in sterile containers, if not indicated otherwise.



#### ***1.1. Collection of blood samples***

For ongoing studies the following volumes of blood will be collected:

- a. For collection of blood on anticoagulant use special sterile tubes, lined with anticoagulant: Sodium citrate, EDTA or Heparin. The minimum volume of blood on anticoagulant is 3.5 mL. After blood collection, invert the tube gently for 5-6 times (or see vacutainers' manufacturer instructions)
- b. Leave the tube for 30 min at room temperature to form the blood clot (or see vacutainers' manufacturer instructions)
- c. For collection of blood on coagulation activator use special sterile tubes lined with coagulation activator. The minimum volume of blood on coagulation activator is 3.5 mL.
- d. After blood collection, invert the tube gently for 5-10 times to completely mix the blood with the anticoagulant and leave the tube for 15 min at room temperature (or see vacutainers' manufacturer instructions)



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- e. Special blood collection tubes for proteomics analysis can be also used. Tubes are spray coated with K2EDTA anticoagulant and a protease inhibitor cocktail, specifically formulated for stabilization of human plasma proteins at the point of collection. The draw volumes is according to the manufacturer’s instructions. Please contact the research team prior stude-design.

### IMPORTANT!!!

- **Check the inscription on the label** of the tube in which the biological sample is collected, the color code of the cap is not universal.
- When collecting, **homogenize the blood tube very well and keep it upright** so that the blood does not touch the rubber cap. If it touches the lid, it will coagulate, even if it is collected on anticoagulant.
- The biological sample is sent for the separation of the blood components, **within maximum 2 hours after the collection and preferably on ice!!!**
- **For each study, all samples will be collected using the same type of tube/anticoagulant and the same procedure .**

### 1.2. Collection of urine samples

- a. For collection of urine sample use sterile plastic containers, without a lining with anticoagulant or coagulation activator lining, preferably in containers for urine samples for culture or coproculture. The minimum volume of urine is 10 mL.

### IMPORTANT!!!

- **Urine is stored at 2-8°C and sent for processing to the laboratory within maximum 2 hours after collection.**

### 1.3. Collection of saliva samples

- a. For collection of saliva samples use sterile containers for urine storage that is further used for urine culture. The minimum volume of saliva is 2 mL. There are also some special saliva collection devices, please contact the research team prior study-design.

### IMPORTANT!!!

- **Saliva is stored at 2-8°C and sent for processing to the laboratory within maximum 2 hours after collection.**



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***1.4. Collection of tissue samples for protein extraction***

- a. For collection of tissue samples further used for protein extraction use protein low binding tubes of 1.5-2 ml. The tube is immersed in liquid nitrogen immediately after collection and further sent out to the laboratory for processing.
- b. Paraffin embedded tissues from retrospective biobanks can be also taken for proteomics analysis. Please contact the research team for a brief discussion prior study-design.

**IMPORTANT!!!**

- The fresh-frozen tissue sample **MUST NOT be thawed**, freeze/thaw steps affect sample quality, and improper transport carries risks of obtaining erroneous results.

**PROCESSING OF BIOLOGICAL SAMPLES FOR PROTEOMICS  
ANALYSIS**

Research Center for Advanced Medicine – Medfuture (UMFIH) can receive and process various biological samples, including liquid biopsies like blood, urine and saliva samples, but also solid biopsies meaning mainly tissue samples. All samples need to be collected in sterile containers, if not indicated otherwise. **Please see [COLLECTION OF BIOLOGICAL SAMPLES FOR PROTEOMICS ANALYSIS](#) link.**



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### 1.1. *Processing of blood samples*

#### a. Isolation and storage of serum samples

- Blood samples are received in the laboratory and registered to be able to keep track of them. The blood **tube lined with coagulation activator is used to isolate the serum**
- The sample is checked for the blood to have the appropriate appearance according to the type of determination to be made after processing of the raw sample
- To separate the serum, centrifuge the tubes at 2000g for 10 min at 4°C (or see vacutainers' manufacturer instructions)
- After centrifugation, the emptied containers are carefully placed in the tube rack and the serum is aliquoted in protein low binding tubes in the desired volume (ex. 300µL)
- The storage of the serum is done at -80°C immediately after aliquoting or after maximum 2h kept at 4°C.

#### b. Isolation and storage of plasma samples

- Blood samples are received in the laboratory and registered to be able to keep track of them. The blood **tube lined with anticoagulant is used to isolate the plasma. Be careful at the tube inscription and use tubes lined with Sodium Citrate, EDTA or Heparin!**
- The sample is checked for the blood to have the appropriate appearance according to the type of determination to be made after processing of the raw sample
- To separate the plasma, centrifuge the tube at 1700g for 10 min at 4°C (or see vacutainers' manufacturer instructions)
- After centrifugation, the emptied containers are carefully placed in the tube rack and the plasma is aliquoted in protein low binding tubes in the desired volume (ex. 300µL)
- The storage of the plasma is done at -80°C immediately after aliquoting or after maximum 2h kept at 4°C.

#### c. Isolation and storage of the population of mononuclear cells (PBMCs), granulocytes (GNCs), trombocytes etc.

- Please contact the research team for a dedicated protocol.



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***1.2. Processing of urine samples***

- Urine samples are transferred in 15 or 50mL tubes and centrifuged at 4000g for 10min at 4°C
- The supernatant is aliquoted into 1.5-2 mL protein low binding tubes (ex. 1000µL) and immediately stored at -80°C until protein extraction.

***1.3. Processing of saliva samples***

- Saliva samples are aliquoted into 1.5-2 mL protein low binding tubes and immediately stored at -80°C until protein extraction.

***1.4. Processing of tissue samples***

- Fresh tissue samples are immediately stored at -80°C in protein low binding tubes until protein extraction, depending on the specifics of the experiment.
- Please contact the research team prior study-design.

**Maximum 2 cycles of freeze-thaw are allowed for good quality preservation for proteomics analysis!**

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