

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

#2 PROCESSING OF BIOLOGICAL SAMPLES

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





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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, set the centrifuge at 3000 rpm for 15 minutes
- After centrifugation, the emptied containers are carefully placed in the tube rack and the serum is transferred to a cryotube or Eppendorf tube
- The storage of the serum is done at -20°C if its processing will be carried out within 60-90 days or at -80°C if the processing will be done after a number of days greater than 90.

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, set the centrifuge at 3000 rpm for 15 minutes
- After centrifugation, the emptied containers are carefully placed in the tube rack and the serum is transferred to a cryotube or Eppendorf tube. From the 3.5 mL sample, approximately 1 mL of plasma is stored, the remaining 2.5 mL of the sample is mixed and processed further
- Plasma storage is done at -20°C if its processing will be carried out within 60-90 days or at -80°C if the processing will be done after a number of days greater than 90
- The sediment (minimum 1 mL) is stored as a whole blood sample and will be used for further determinations. The whole blood is stored at -80°C.



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c. Isolation and storage of the population of mononuclear cells (PBMCs) and granulocytes (GNCs)

The anticoagulant blood tube contains 3.5 mL of sample – 1 mL is stored as plasma, 1 mL is stored as whole blood, and the remaining of a minimum 1.5 mL is processed as follows:

- A volume of blood is mixed with a volume of phosphate buffer (PBS 1X) (ex. 1.5 mL blood + 1.5 mL PBS)
- Into a sterile 15 mL tube, pipette a minimum of 3 mL of FICOLL (if the available blood sample is greater than 3 mL, then keep the ratio of one volume of Ficoll to one volume of blood – 1 Ficoll: 1 Blood: 1 PBS 1X)
- **Carefully pipette the blood/PBS mixture over the FICOLL, drop by drop, keeping the tube tilted at 45 degrees**
- The sample is centrifuged for 30 minutes, at room temperature, 560xg, with slow acceleration and no assisted braking
- Collect the "Buffy Coat" from the interface between FICOLL and the resulting plasma, and transfer to a new 15 mL tube. The layer from the interface between FICOLL and the red sediment is collected from under the FICOLL and transferred to a new tube.
- Fractions of PBMCs and GNCs will be subjected to erythrocyte lysis for 15 minutes with Lyza solution prepared in the laboratory or commercially available, after which the samples are centrifuged for 10 minutes, at 4°C, at 600xg
- The resulting sediment is washed twice with phosphate buffer (PBS1X) by centrifugation, centrifuged for 10 minutes, at 4°C, at 600xg
- Cells isolated in this way are stored as appropriate:
 - either in TRIZOL (800 µL TRIZOL/sample) and quickly frozen in liquid nitrogen.
 - either in a mixture Fetal Bovine Serum (FBS) with 10% DMSO
- Storage is carried out in sterile 1.5 mL tubes, at -80°C.

1.2. Processing of saliva and cerebrospinal fluid (CSF) samples

- a. Saliva and CSF samples are aliquoted into 1.5 mL tubes and immediately stored at -80°C.





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1.3. Processing of tissue samples

- a. Tissue samples are immediately stored at -80°C in labeled tubes. Afterwards, different protocols will be applied, like DNA, RNA or protein isolation, depending on the specifics of the experiment.

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