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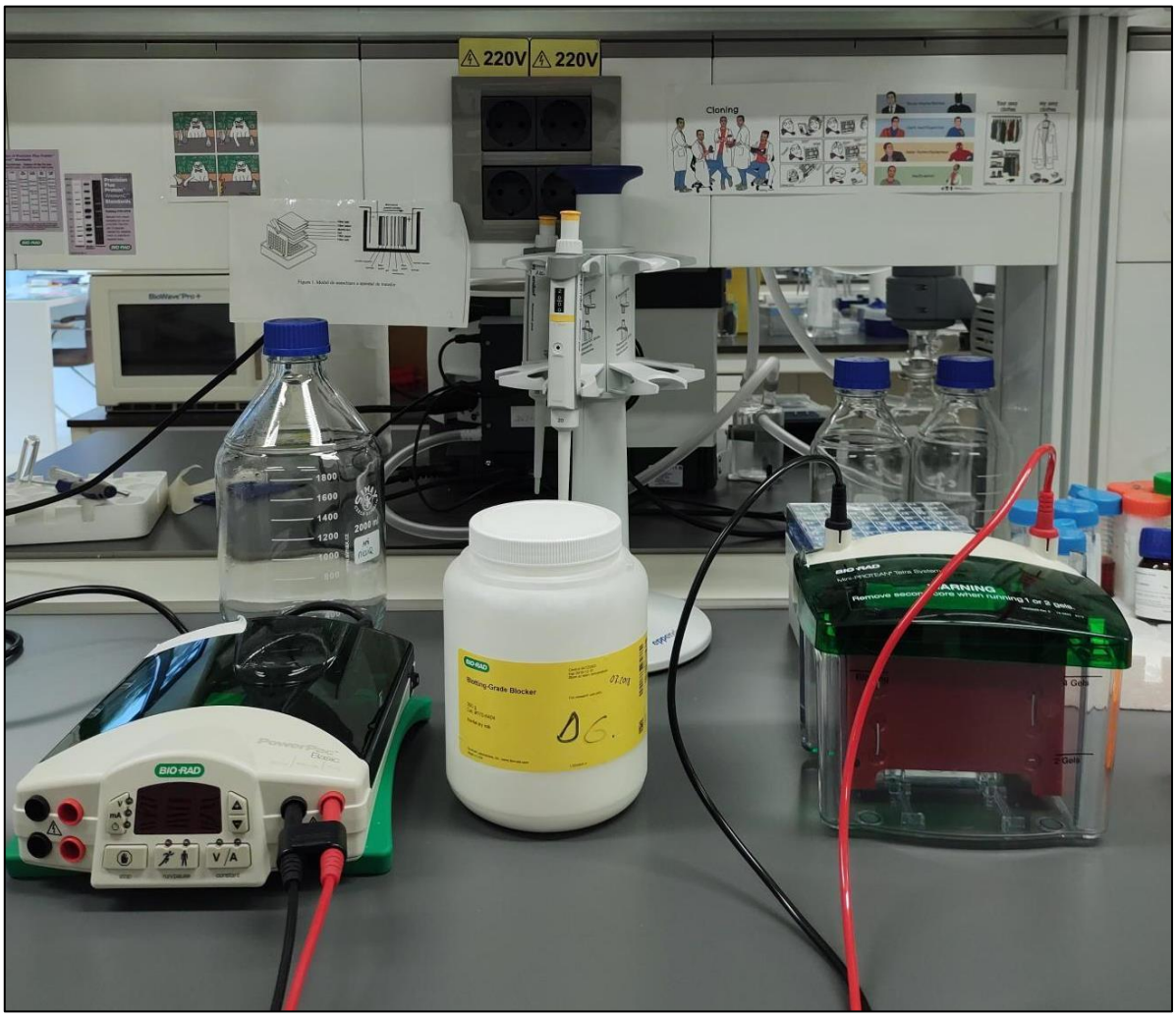
The Education, Scholarships, Apprenticeships and Youth Entrepreneurship Programme – EEA Grants 2014-2021

#6 PROTEIN EXTRACTION FROM THE ORGANIC PHASE OF THE TRIREAGENT SAMPLES

Research Center for Advanced Medicine – Medfuture (UMFIH) can perform molecular assays based on RNA, DNA or proteins in order to evaluate the expression and the modification of these molecules after different treatments or stimuli or as potential biomarkers for diseases. More info can be found at: https://medfuture.umfcluj.ro/.



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Protein extraction from biological samples (e.g. cell and tissue samples) is the incipient protocol in all assays involving protein analysis between different types of samples (e.g. normal and pathological tissue, control and treatment samples) in order to evaluate the molecular changes that take place at translational level. The proper execution of this protocol is vital for the ongoing of the next analysis, the majority of them requiring strict sample standards like protein integrity and purity.

a. Add 0.3 volumes of Ethanol 96% over the remaining TRIREAGENT
b. Shake and centrifuge for 5 minutes at 2000xg at 4°C, for DNA precipitation
c. Transfer the mixture into a 2 mL tube and add 1.5 volumes of isopropanol
d. Mix the content and incubate at room temperature for 10 minutes
e. Centrifuge for 10 minutes at 12000xg at room temperature and remove the supernatant
f. The sediment is washed with 0.5 mL of 0.3 M Guanidine HCl solution in 96% ethanol, shaking the sample very well and then centrifuging at 7500xg, for 5 minutes, at room temperature (the step can be repeated up to three times)
g. The sediment is washed with Ethanol 96% and incubated at room temperature for 20 minutes, then centrifuged at 7500xg, 5 minutes, at 4°C
h. The supernatant is removed, and the sediment is left for 10 minutes at room temperature to allow fot all the ethanol to evaporate
i. The sediment is solubilized in a 1:1 solution of SDS/Urea in 1M TrisHCl as follows: solution containing 1% SDS (1mL SDS10% + 9mL 1M Tris HCl) and 8M Urea (2.4g Urea in 5mL 1M Tris HCl)
j. The samples are sonicated for 5 cycles of 15 seconds, 80% amplitude and incubated on ice for 30 seconds
k. The obtained solution is stored at -80°C.

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