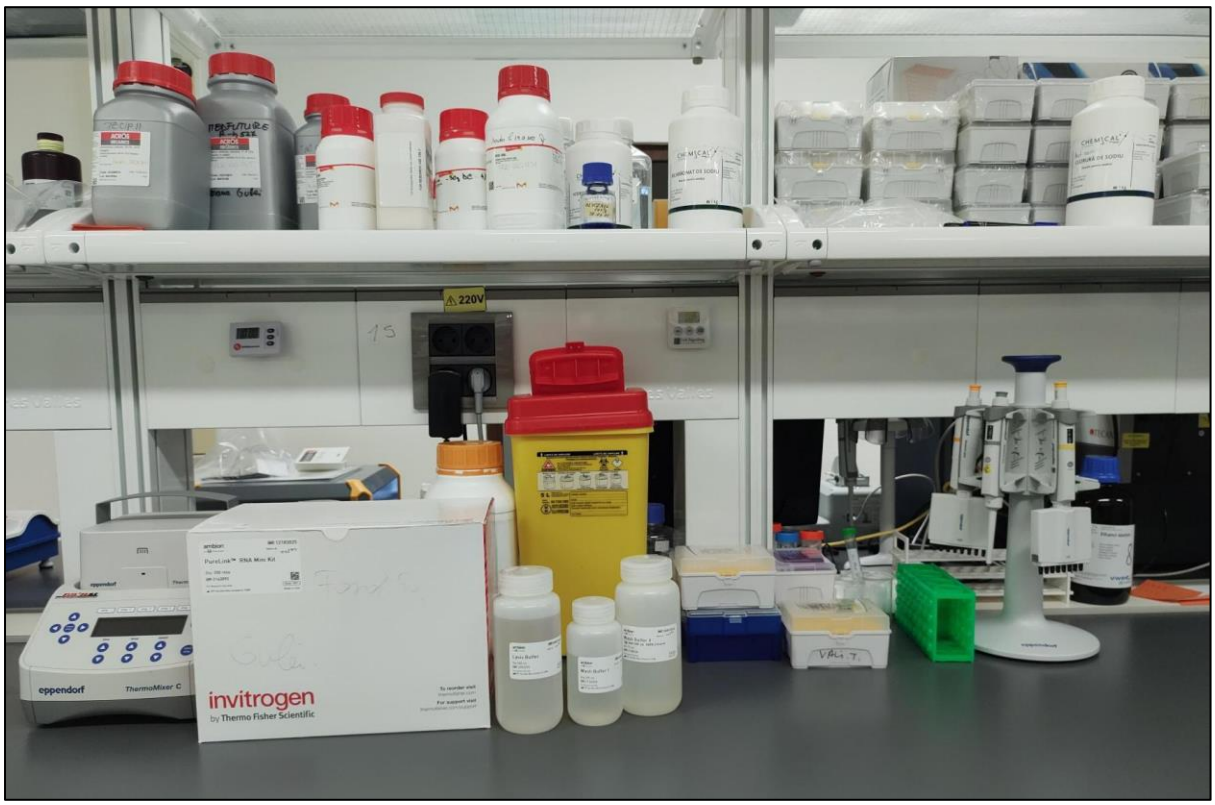
Iceland Liechtenstein Norway grants "Working together for a green, competitive and inclusive Europe"

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

#5 RNA Extraction (from TRIREAGENT)

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/.



RNA extraction from biological samples (e.g. cell and tissue samples) is the incipient protocol in all assays involving RNA 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 transcriptional 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 RNA integrity and purity.

a. Add 800 µL of TRIREAGENT to the sample of interest (cell sediment or tissue) and flash freeze the sample using liquid nitrogen; store at -80°C
b. Before extraction, the sample in TRIREAGENT is brought to room temperature
c. Add 160 µL of Chloroform and vortex for 10 seconds
d. The sample is incubated for 5 minutes at room temperature
e. After incubation, the tubes are centrifuged at 13.000 RPM, 20 minutes, at 4°C
f. The aqueous phase (clear layer) containing RNA, is transferred to a separate tube. The rest (the organic phase, pink, containing proteins and the white film containing DNA) is stored at -80°C
g. Add 500 µL of pure Isopropanol to the collected aqueous phase, vortex and incubate for 15 minutes at room temperature
h. The samples are centrifuged at 13,000 RPM, 15 minutes, at 4°C
i. The isopropanol is carefully removed without disturbing the cell pellet
j. Add 1 mL of Ethanol 75%. DO NOT VORTEX, DO NOT SHAKE!!!
k. Centrifuge the samples for 5 minutes at 10.000 RPM, at room temperature
l. Carefully remove all the ethanol and leave the samples in the sterile biological hood, with the lids open to let the rest of the alcohol to evaporate
m. Add 25-30 µL DNAse/RNAse-free ultrapure water to the tube and store at -80°C
n. Optional before storage: Measure the quality and quantity of the RNA using a NanoDrop instrument.

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