UNIVERSITY of LIFE SCIENCES in Lublin
FACULTY OF VETERINARY MEDICINE
DEPARTMENT OF BIOCHEMISTRY
20-033 Lublin, Akademicka 12
Phone +48 81 445 66 08
www.biochwet.up.lublin.pl

Nucleic acids

Task 1. Extraction of RNA from yeast

The aim of the experiment is to extract RNA from yeast cells and the hydrolysis of extracted RNA to obtain each component of nucleotide.

Yeast homogenization and RNA extraction. Weigh 5 grams of yeast on piece of paper or aluminium foil, put it into a mortar. Add about half a teaspoon of glass sand and grind the mixture using pestle set about 5 minutes. Next start to add drop by drop 2.5 cm^3 of 2M NaCl, still continuing grinding during next 3 minutes.

Put the obtained mixture into long glass tube and shake briskly during 10 minutes.

Now, insert the tube with homogenic mixture into boiling water bath for 7 minutes. To prevent cracking the tube, mix the mixture using glass baguette. Next, cool the tube under the stream of tap water and transfer the mixture to a plastic tube.

Centrifuge the tube using the force 2000 x g during 10 min.

Transfer the obtained supernatant to new and clean plastic tube, add to it drop by drop 3 cm^3 of chloric acid, and wait 10 minutes for the formation of precipitate.

Next, centrifuge this tube using the force 2000 x g during 10 min. After it, remove the supernatant, add to the tube 6 cm^3 of distilled water and dissolve the precipitate by mixing it thoroughly. Transfer this solution to new long, glass tube.

Hydrolysis of obtained RNA. Add to the glass tube including RNA solution 3 cm^3 of 10M sulfuric acid. Wait 30 minutes. Next, make the tests for the detection of pentose, purine base and phosphate residues.

Task 2. Detection of each component of nucleotide

The aim of this part of experiment is to detect each component of nucleotide to confirm its composition. Note the results in the table below.

Detection of pentose. Take 1 cm^3 of the acidic hydrolysate achieved in task 1 to new glass tube and add 1 cm^3 of Bial reagent

or add 1 cm^3 of Tollens reagent. Heat the tube carefully over the fire of burner.

Now, to make remaining tests, the acidic hydrolysate must be neutralized by adding 2 cm^3 of 2M NH_3.aq.

Detection of purine bases. Take 2 cm^3 of neutralized hydrolysate to new glass tube, add 5 drops of 0.1 M $AgNO_3$ still mixing. Next, add 1 cm^3 of 2 M NH_3.aq. Purine bases form silver salts in ammonium solution, and then precipitate.

Detection of phosphate residues. Take 1 cm^3 of neutralized hydrolysate to new glass tube, and add 0.5 cm^3 of concentrated solution of HNO_3 and 1 cm^3 of ammonium molybdate. Heat the tube carefully over the fire of burner and boil the content of the tube about 3 minutes.

Test	**Result**
Detection of pentose	
Detection of purine bases	
Detection of phosphate residues	