ÚSTAV LÉKAŘSKÉ BIOCHEMIE A LABORATORNÍ DIAGNOSTIKY 1. LF UK A VFN

DNA Isolation

Practical lesson on medical biochemistry

General Medicine

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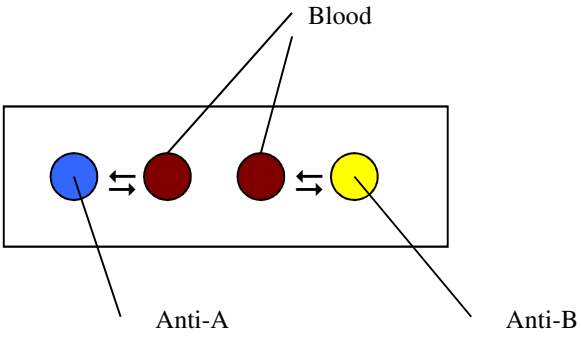
Task 1: Determination of blood group by hemagglutination test

Procedure:

1. Disinfect the belly of the finger from which the blood sample is to be taken. Capillary blood is usually taken from belly of the 3rd or 4th finger, from the left hand in right-handers.
2. Sting the belly of the finger with a sterile needle and apply two droplets of blood close to centre of a glass slide.
3. Drop antibody anti-A on the left side of the slide and anti-B on the right side.



4. Mix the blood droplets with each antibody (use pipette tips) and evaluate after approximately 1 min.

Evaluation:

If the red blood cells carry antigen A or B, they agglutinate (aggregate) in the presence of corresponding antibody.

Task 2: Isolation of DNA from buccal smear using phenol-chloroform

Reagents:

Homogenization buffer 2× conc. (EDTA 80 mmol/l, Tris-HCl 80 mmol/l pH 7.65, NaCl 400 mmol/l, sterilized in autoclave)
Sodium dodecylsulfate 10 g/l, sterilized by filtration
Physiologic saline solution (NaCl 150 mmol/l) sterilized in autoclave
Proteinase K 20 mg/ml

Phenol equilibrated with Tris, pH 8, stabilized with 0.1% 8-hydroxychinoline 

Chloroform-isoamyl alcohol 24:1 

Isopropanol 

Ethanol (pure) 70% 

TE buffer (Tris-HCl 10 mmol/l, EDTA 1 mmol/l, pH 7.6, sterilized in autoclave)
Sodium acetate 3 mol/l with glycogen 4 g/l, sterilized in autoclave

Tools:

Sterile swabs, sterile micro tubes (1.5 ml), sterile tissue cloths, sterile tips of all sizes, gloves

Procedure:

1. Wipe epithelial cells of buccal mucosa with a sterile swab. Wash the swab in 1 ml of physiologic saline solution in a micro tube and centrifuge the obtained suspension (1 min) in a microcentrifuge.
2. Discard the supernatant.
3. Resuspend the pellet in 300 µl of homogenization buffer and mix thoroughly by vortexing.
4. Add 300 µl SDS and mix by repeated pipetting in and out.
5. Add 1 µl of proteinase K and mix by repeatedly turning the tube upside down.
6. Incubate for 45 minutes in thermo block at 50°C, shaking 300 rpm.

DNA extraction using phenol-chloroform

Work in the fume hood!
Use gloves!

1. Prepare the mixture of phenol-chloroform-isoamyl alcohol (25:24:1): In a glass tube mix 1.2 ml of phenol with 1.2 ml of chloroform-isoamyl alcohol (24:1).
2. To the cell lysate (600 µl) from the previous procedure add 600 µl of the mixture phenol-chloroform-isoamyl alcohol (25:24:1). Vortex for 1 minute (a milky emulsion should be formed), and then centrifuge in the bench-top micro-centrifuge for 3 minutes.
3. Transfer the upper aqueous phase to another clean micro tube (aspirate the solution carefully to avoid contamination with proteins precipitated at the boundary between the aqueous and organic phase).
4. To the aqueous phase from step 3 add 600 µl of the mixture phenol-chloroform-isoamyl alcohol (25:24:1). Mix by intense turning the micro tube upside down (a milky emulsion should be formed), and then centrifuge in the bench-top micro-centrifuge for 3 minutes.
5. Transfer the upper aqueous phase to another clean micro tube.
6. To the aqueous phase from step 5 add 600 µl of chloroform-isoamyl alcohol (24:1), mix briefly by turning the tube upside down and centrifuge approximately 10 s.
7. Transfer the aqueous phase to a clean micro tube, add 70 µl of sodium acetate solution with glycogen and mix by turning the tube upside down.
8. Add 600 µl of isopropanol and mix by turning the tube upside down. Then allow to stand 15 minutes at room temperature.
9. Rotate in the large centrifuge for 10 minutes at 10,000 g.
10. Discard the liquid contents (the pellet with isolated DNA will adhere to the wall near the bottom of the tube) and dry the test tube by pushing its orifice upside down onto a piece of sterile tissue cloth.
11. Add 1 ml of 70% ethanol and centrifuge for 10 min.
12. Discard the supernatant and again dry the test tube with a sterile tissue cloth. Let the residual ethanol to evaporate by leaving the tube open for several minutes at 80 °C.
13. Add 20 µl of TE buffer, mix several times by repeated pipetting in and out and briefly spin in the micro-centrifuge.

Task 3: Determination of concentration and purity of DNA

Procedure:

1. Take a spectrophotometric capillary out from the box. Touch the capillary only at the end that will not be used for measurement.
2. Insert one end of the capillary to the sample in micro tube and allow the solution to rise at least 2/3 of the capillary length.
3. Close the capillary by pressing the sample end it into the special clay. The resulting stopper should be about 2 mm high.
4. Place the capillary into a special holder in the spectrophotometer.
5. Measure the absorption spectrum between 240 and 330 nm against the TE buffer. Record the absorbances at 260, 280 and 320 nm.
6. Evaluate the purity of isolated DNA and estimate its concentration using the formula in report form.