Z48 - Polyacrylamide Gel Electrophoresis (PAGE)

Physics Laboratory II – academic year 2017/2018
Faculty of Physics, Astronomy and Applied Computer Science, Jagiellonian University

During classes you will have opportunity to learn the most popular method used in molecular biology for qualitative and quantitative protein analysis.
Polyacrylamide gel electrophoresis (PAGE) in denaturing conditions has been developed by Urlich K. Laemmli (1970), where incorporated detergent sodium dodecyl sulphate (SDS) into discontinuous denaturing buffer system was used to analyze bacteriophage T4 protein profile and molecular mass. Since this time the paper by Leammli has been cited more than 300 thousand times and the SDS-PAGE method were applied in many modifications for the basic and advanced protein analysis, including protein cleaning for mass spectrometry (MS).

Preparatory questions

1. Viscosity of a fluid: definition of absolute and kinematic viscosity, their main relations, units, how to calculate viscosity coefficient η (μ)? [1]
2. Viscosity measure and Stockes' law [2].
3. Ohm's law and conductivity definition.
4. Express the equitation of the movement of electric particle (q) in an electrostatic field (E).
5. What electrolytes are? Give an example of inorganic and organic ones [3, 4].
6. What electrolysis is? Explain the conditions of this process.
7. Aminoacid structure [4].
8. Give an example of aminoacid electrolysis in an acidic medium and a basic medium.
9. Why proteins migrate towards anode during electrophoresis?
10. Give an example of organic polymers as electrophoresis carriers.
11. What is relative electrophoretic mobility (Rf) of protein bands, how it depends on a carrier and protein mass? [4]

Computational assignments

1. Giving experimentally achieved relative protein mobility (Rf) calculate protein molecular mass (kDa) for analyzed protein samples, in different electrophoretic conditions: PAGE gel concentrations 10%, 2% i 15%.
2. Use a standard protein mass curve appointed for a protein standard Precision Plus Protein Dual Color Standards (BioRad) and Image Lab (Bio-Rad) Origin software (Fig. 1).

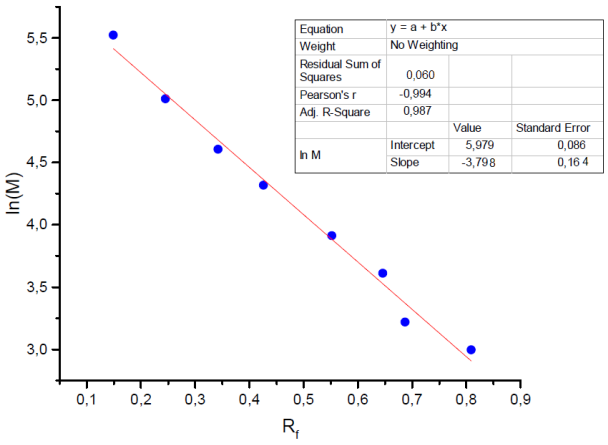

Figure 1: An example of (Rf) for a protein standard in 10% PAGE electrophoresis (courtesy Irena Rodzoń, a 3^{rd} year Biophysics student, a class report 2015)

Apparatus and materials

Chemicals:

1. Demineralised water
2. 96% denatured ethyl alcohol (Linegal Chemicals, nr kat. LL-00012)
3. 40% acrylamide (Bio-Rad, nr kat. 1610140)
4. 2% Bisacrylamide (Bio-Rad, nr kat. 1610142)
5. 10% SDS (Bio-Rad, nr kat. 1610416)
6. TEMED (Bio-Rad, nr kat. 1610800)
7. 10% Ammonium persulfate (APS) (Bio-Rad, nr kat. 161-0700)
8. 1,5 M Tris-HCl Buffer, pH 8,8 (Bio-Rad, nr kat. 1610798)
9. 0,5 M Tris-HCl Buffer, pH 6,8 (Bio-Rad, nr kat. 1610799)

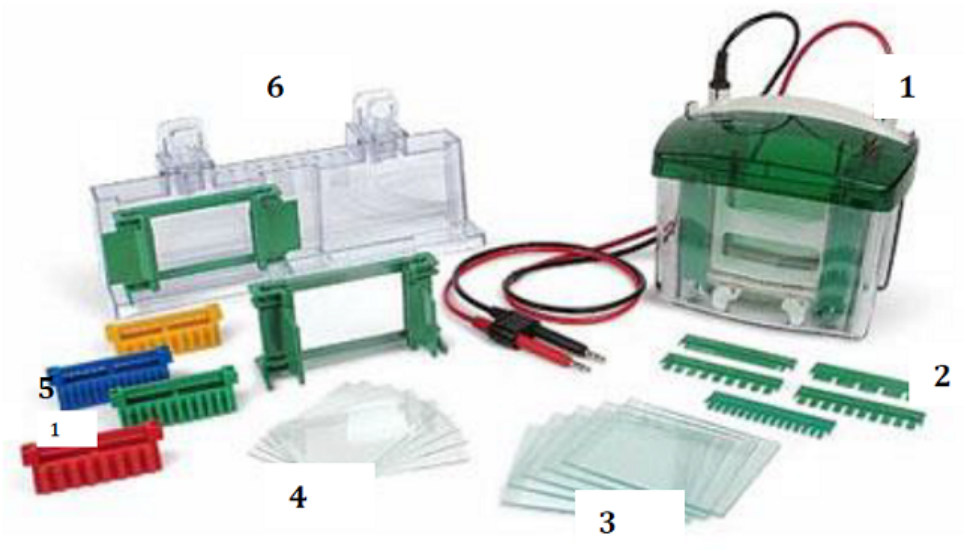

Figure 2: Typical vertical (PAGE) electrophoresis equipment (Bio-Rad, Mini-PROTEAN® Tetra Cell Systems). 1. Electrophoresis tank with a cover, electrodes and wires, 2. Combs needed for wells coasting in PAGE gels, 3. Casting plates with 1mm spacers, 4. Cover plates, 5. Protein loading stands, 6. Casting stand.

10. Laemmli premixed protein sample buffer for SDS-PAGE (Bio-Rad, nr kat. 161-0737)
11. β-mercaptoethanol (Bio-Rad, nr kat. 161-0710)
12. 10x Electrophoresis Buffer Tris/Glycine/SDS (Bio-Rad, nr kat. 161-0732)
13. Precision Plus Protein^{TM} Dual Color Standards (Bio-Rad, nr kat.1610374).
14. Automatic pippets, pipette tips, eppendorf tubes 1,5 mL, Falkon tubes 15 mL i 50 mL

Experiment

1. Check and prepare chemicals and materials needed for SDS-PAGE electrophoresis.
2. Check prepare equipment needed for SDS-PAGE electrophoresis.
3. PAGE gel casting (see a movie on You tube) [5]:
   - Preparation of running gel
   - Preparation of stacking gel
4. Sample denaturation and preparation for gel loading.
5. Electrophoresis.
6. Gel fixxing.
7. Gel staining.
8. Gel analysis Image lab.

Data analysis

1. Calculate protein masses by means of Origin and Image Lab software.
2. Compare protein masses calculated by means of Origin and Image Lab software with protein masses given by manufacturer.
3. Perform and present densitometry analysis for every protein lane (sample) and present protein mass as (kDa) (Fig. 3)
4. Calculate measurement uncertainty (standard deviations) for different PAGE concentrations for the same samples

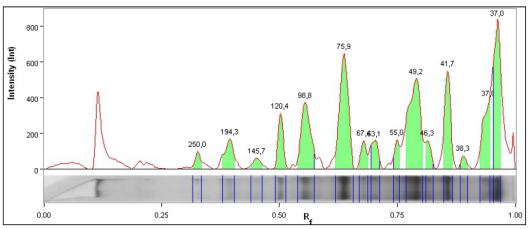

Figure 3: Example of densytometry analyzis of protein lysate from extracellular microvesicsle sample. 10% SDS-PAGE.

Safety rules

1. Please have lab clothes, classes and gloves to protect yourself.
2. Do not touch an electrophoresis tank whet it is switch on.

References

[1] *Instruction for Rotary Viscosimetry classes* – 1st Laboratory in Physics (M17)

[2] *Instruction for Stockes' viscosity coefficient classes* – 1st Laboratory in Physics (M16)

[3] A Guide to Polyacrylamide Gel Electrophoresis and Detection. BioRad pp. 9-16

[4] Lubert Stryer, Tymoczko John L, Berg Jeremy M., *Biochemistry: A Short Course*, 3rd Edition ISBN-13: 978-1464126130

[5] https://www.youtube.com/watch?v=pnBZeL8nFE oor another one