

Comenius University in Bratislava, Faculty of Medicine
Institute of Medical Chemistry, Biochemistry and Clinical Biochemistry

LABORATORY PROTOCOL GM-WS - 1st seminar
Determination of lactate dehydrogenase (LD) activity

Name, group:	Date:
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Principle:

Lactate is oxidized to pyruvate, hydrogen atoms are transferred to coenzyme NAD⁺ and NADH+H⁺ is being created. Artificial acceptor of electrons from NADH+H⁺ is ferrifenantroline complex (Fe³⁺), which is reduced to ferofenantroline (Fe²⁺). Ferofenantroline is red colored compound and intensity of red color is proportional to activity of LD.

Procedure:

sample	A	B	C	reference sample
reaction mixture (in dispenser)	2.5 ml	2.5 ml	2.5 ml	2.5 ml
substrate - lactate (in dispenser)	0.5 ml	0.5 ml	0.5 ml	0.5 ml
serum A (diluted 1:10)	0.5 ml	---	---	---
serum B (diluted 1:10)	---	0.5 ml	---	---
serum C (diluted 1:10)	---	---	0.5 ml	---
water	---	---	---	0.5 ml
We let the samples stand for 10 min at laboratory temperature.				
TCA (stops the reaction)	0.5 ml	0.5 ml	0.5 ml	0.5 ml
We mix the samples and measure absorbance at 510 nm.				

Calculation:

sample	A	B	C
absorbance			
μmol from calibration curve			
μmol/l			
μkat/l			
correction for dilution			

Reference values of LD activity in serum: **4.2 – 6.0 μkat/l**

Conclusion:

Literature:

- Practical exercises in biochemistry (Asklepios, 1993) – Chapter 2 - Theoretical part (except picture on page 16 - Fig. 2.4) and Chapter 3 - Theoretical part.

Literature for next week:

Lippincott's – Chapter 8, part II - Regulation of Metabolism; Figure 18.28 at the end of Chapter 18 about steroid hormone action; Chapter 17, part III, letter D: Phosphatidyl inositol including Figure 17.8.

- You can also take a look at the following Youtube videos:

https://www.youtube.com/watch?v=Le_f5cxpD4w

<https://www.youtube.com/watch?v=0nA2xhNiAow>

<https://www.youtube.com/watch?v=NaOBRvAFiJQ>

<https://www.youtube.com/watch?v=2bbBrpgeheY>

<https://www.youtube.com/watch?v=lsYBeFqEwzk>

https://www.youtube.com/watch?v=larIwx_9ePU