P2. Atlas of Enzymes

Equipment

Item	Quantity	Label
Eppendorf tubes rack	2	
Micropipette, 10-100 μL	1	
Micropipette tips	40	
Tips box	1	
96-well plate	2	

Chemicals

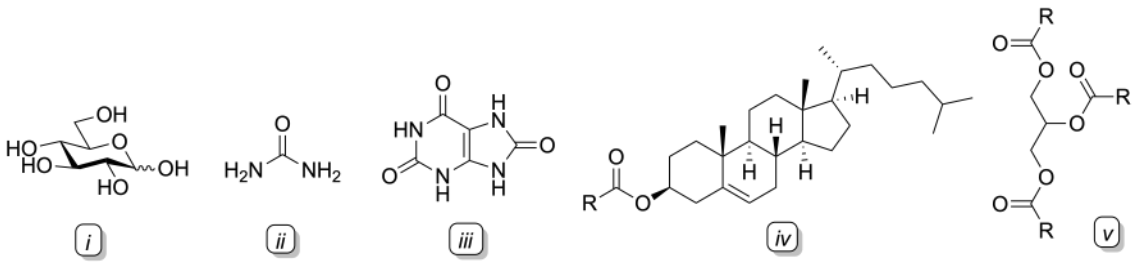
Name	State	Concentration	Quantity	Placed in	Label
Part A					
Mixture **E1**	aq	-	4 mL	Eppendorf tube, 5 mL	**E1**
Mixture **E2a**	aq	-	4 mL	Eppendorf tube, 5 mL	**E2a**
Mixture **E2b**	aq	-	4 mL	Eppendorf tube, 5 mL	**E2b**
Mixture **E3**	aq	-	4 mL	Eppendorf tube, 5 mL	**E3**
Mixture **E4**	aq	-	4 mL	Eppendorf tube, 5 mL	**E4**
Mixture **E5**	aq	-	4 mL	Eppendorf tube, 5 mL	**E5**
Substrates solutions **S1**–**S10**	aq	-	0.5 mL	Eppendorf tube, 0.5 mL	**S1**–**S10**
Part B					
Substrates solutions **M1**–**M3**	aq	-	0.5 mL	Eppendorf tube, 0.5 mL	**M1**–**M3**
Part C					
Substrates solutions **W1**–**W7**	aq	-	0.5 mL	Eppendorf tube, 0.5 mL	**W1**–**W7**

11 % of total									
Question	2.1	2.2	2.3	2.4	2.5	2.6	2.7	2.8	**Total**
Points	50	2	4	1	5	9	35	2	108



Atlas is a smooth single-faced fabric woven from natural silk. Atlas is not just a simple fabric—it is a woven chronicle of the unique Uzbek culture and identity.

Enzyme mixtures are used for qualitative and quantitative analysis of organic substances in biological fluids (plasma, urine, etc.). **E1**–**E5** can contain one or several enzymes. These also contain all the necessary reagents (buffer and other components). **E1**–**E5** are designed for the selective determination of *i) glucose*, *ii) urea*, *iii) uric acid*, *iv) cholesteryl ester,* and *v) triglyceride.*



All reaction mixtures in this problem are prepared in a 96-well plate using a micropipette.

The instructions below apply when performing the reaction between any substrate solution with any enzyme mixture **E1**–**E5**:

1. For all reaction tests except those with **E2**, **add** 20 μL of the substrate solution to 100 μL of the enzyme solution and **incubate** for approximately 5 min at room temperature.
2. For mixture **E2** (composed of separate solutions **E2a** and **E2b**), **add** 20 μL of substrate solution to 100 μL of **E2a** solution. **Incubate** for approximately 5 min at room temperature. **Add** 100 μL of the **E2b** solution to this mixture and **continue** to incubate for approximately another 5 min at room temperature.
3. If the tested solution contains the specific substrate, the mixture shows a colour distinct from the other solutions without the specific substrate.

Part A

You are given the following substances in the 10 solutions **S1**–**S10**. Each solution **S1**–**S10** contains only two substances out of the five substances *i*–*v*. **S1**–**S10** contain different combinations of substances *i*–*v*. The composition of **S1**–**S5** is:

- **S1**: *i* + *ii*
- **S2**: *i* + *iv*
- **S3**: *ii* + *iii*
- **S4**: *iii* + *v*
- **S5**: *iv* + *v*

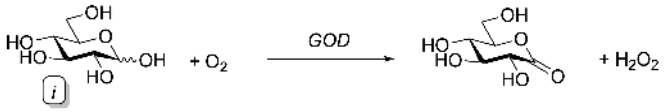
Perform the tests of each solution **S1**–**S10** with each enzyme mixture **E1**–**E5**. The tubes in your rack might not necessarily be arranged in numerical order. Some substrates could appear as cloudy solutions or suspensions.

Q2.1	**Tick** the box with the corresponding colour change for each of the enzyme mixtures **E1**–**E5** with substrates **S1**–**S10**, relative to the initial colour of **E1**, **E2a**, **E3**, **E4**, and **E5**. The colour codes are given below: **N** - no colour change **Y** - change to yellow **G** - change to green **B** - change to black **PR** - change to pink/red	50 pt

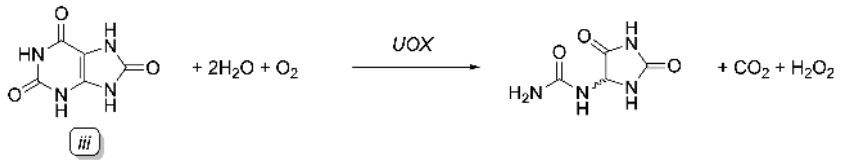
Q2.2	**Tick** the box for the enzyme–substrate pair(s) that gave a positive result.	2 pt

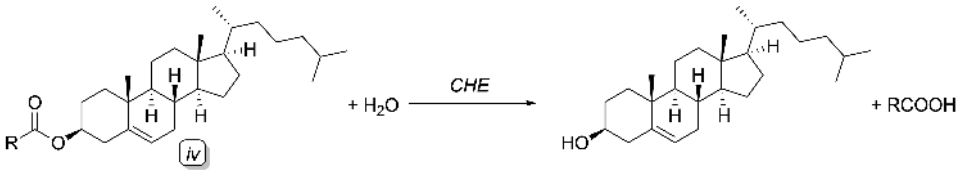
Q2.3	**Tick** the boxes for the correct substrates present in **S6**–**S10**.	4 pt

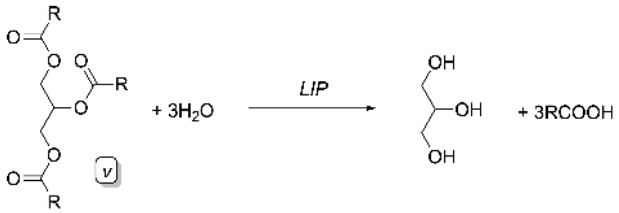
The reaction scheme of enzymes, substrates *i*–*v*, and additional components of mixtures **E1**–**E5** are given below:

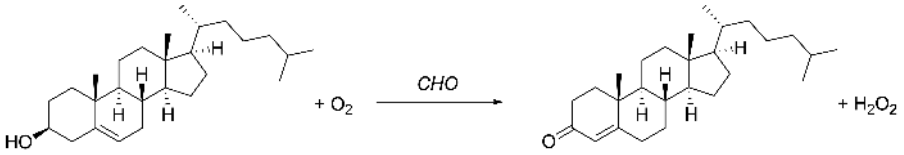


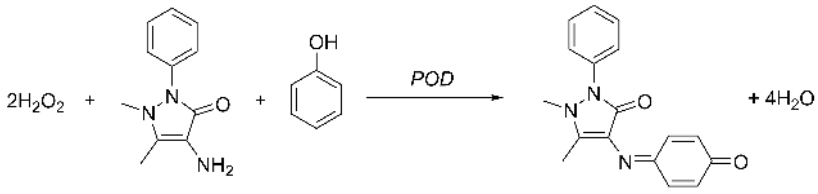


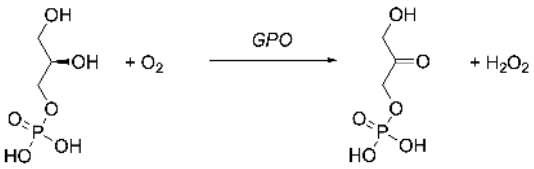


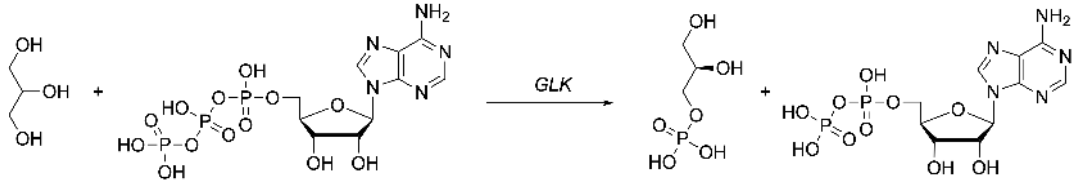

iv
+ H_2O
CHE
+ RCOOH

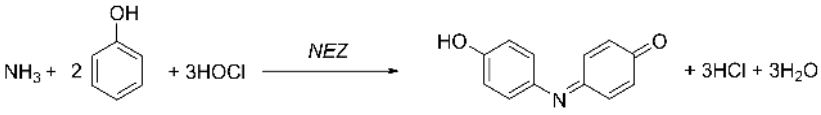

v
+ $3H_2O$
LIP
+ 3RCOOH


+ O_2
CHO
+ H_2O_2


$2H_2O_2$ +
+
POD
+ $4H_2O$


+ O_2
GPO
+ H_2O_2


+
GLK
+


NH_3 + 2
+ 3HOCl
NEZ
+ 3HCl + $3H_2O$

All of the reactions above are balanced. Abbreviations:

- Glucose oxidase – *GOD*
- Urease – *URE*
- Uricase – *UOX*
- Cholesterol esterase – *CHE*
- Lipases – *LIP*
- Cholesterol oxidase – *CHO*
- Peroxidase – *POD*
- Glycerol-3-phosphate oxidase – *GPO*
- Glycerol kinase – *GLK*
- Non-enzymatic reaction – *NEZ*

Q2.4 Intensely coloured products are formed only in two reactions from the list above. **Tick** the boxes for the reactions that give coloured products. 1 pt

Q2.5 **Tick** the components that are present in reaction mixtures **E1**–**E5**. 5 pt

Part B

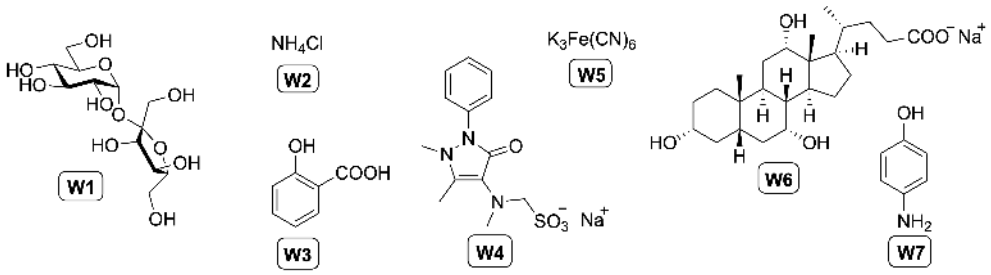
Enzymes **E2**, **E4**, and **E5** give positive tests with substrates **F**, **G**, and **H**, respectively. Solutions **M1**, **M2**, and **M3** contain all of the substrates **F**, **G**, and **H** in different concentrations. For solutions **M1**–**M3**, after approximately 5 min of enzymatic reaction, the colour intensity of the reaction mixture is linearly dependent on the initial concentration of the substrate. Assume that approximately 5 min is enough to achieve complete substrate conversion.

Perform the tests of each solution **M1**–**M3** with each enzyme mixture **E2**, **E4**, and **E5**. Some substrates could appear as cloudy solutions or suspensions.

Q2.6 **Arrange** **M1**–**M3** in increasing order of concentrations for each of **F**, **G**, and **H** substrates based on the performed reactions. 9 pt

Part C

Some or all of the substances **W1**–**W7** can react with components of mixtures **E1**–**E5** and interfere with the accurate determination of their native substrates. Substances **W1**–**W7** are given below:



Perform the tests of each substance **W1**–**W7** with each enzyme mixture **E1**–**E5**. Some substrates could appear as cloudy solutions or suspensions.

Q2.7	**Tick** the box with the corresponding colour change for each of the enzyme mixtures **E1**–**E5** with substances **W1**–**W7**. Use the same colour codes as in **Q2.1**. Compare the colour of the reaction mixtures to the initial colour of **E1**, **E2a**, **E3**, **E4**, and **E5**.	35 pt

Q2.8	**Write down** the balanced reaction equation(s) that describe(s) the positive test(s) in **Q2.7**.	2 pt