# 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

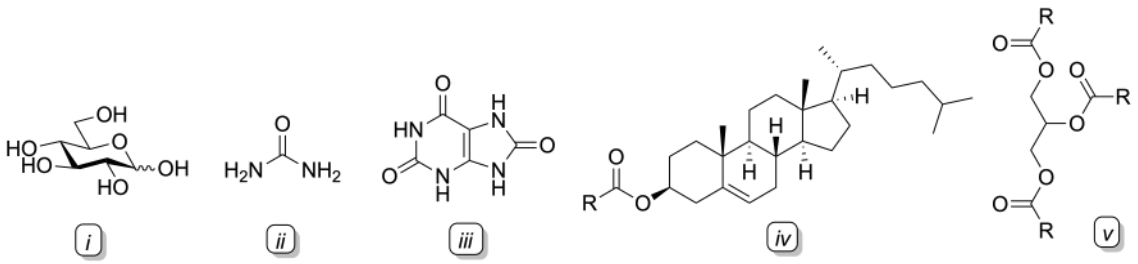
| Name | State | Concentration | Quantity | Placed in | Label |
|---|---|---|---|---|---|
| **Part A** | | | | | |
| Mixture **E1** | aq | - | 4 mL | Eppendorf tube, 5 mL | **E1** |
| Mixture **E2a** | aq | - | 4 mL | Eppendorf tube, 5 mL | **E2a** |
| Mixture **E2b** | aq | - | 4 mL | Eppendorf tube, 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.

# Experiment

58th International Chemistry Olympiad Uzbekistan 2026

# Q2-4

English (Official)

## Part A

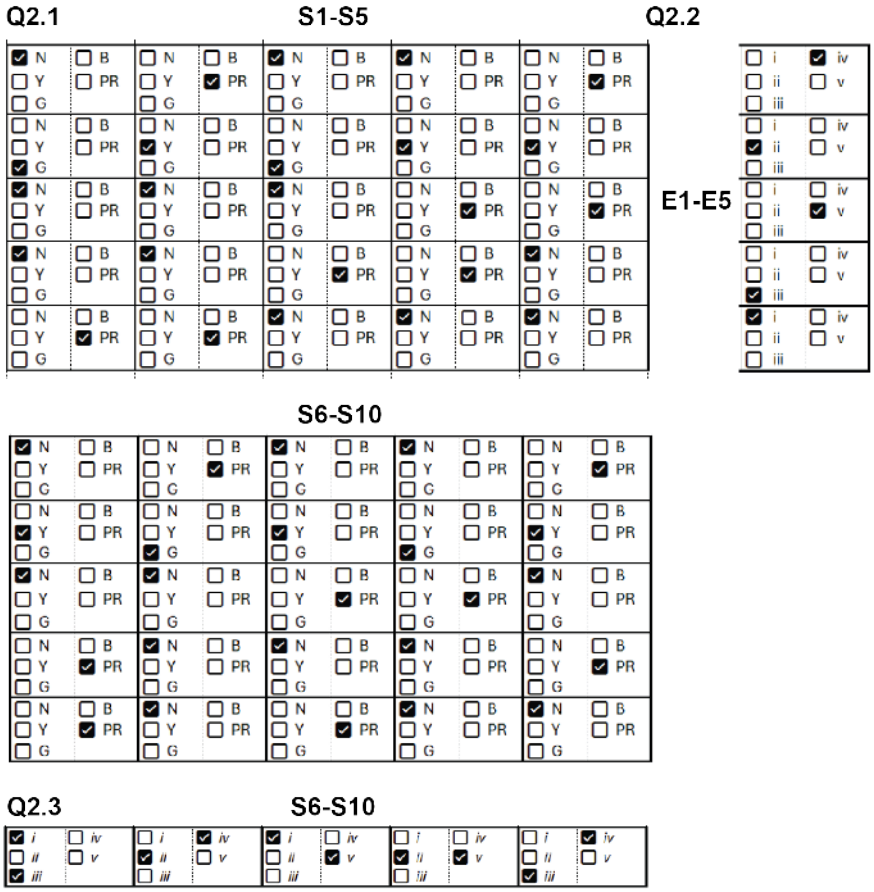
You are given the following substances in the 10 solutions **S1**–**S10**. Each solution **S1**–**S10** contains only two substances out of the five substances *i*–*v*. **S1**–**S10** contain different combinations of substances *i*–*v*. The composition of **S1**–**S5** is:

- **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.

**SOLUTION:**

**Q2.1–Q2.3**



**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: 50 pt

**N** - no colour change
**Y** - change to yellow
**G** - change to green
**B** - change to black
**PR** - change to pink/red

**SOLUTION:**

For each correct choice per enzyme per substrate colour change: 1 pt.
0 pt if there is more than one choice per enzyme per substrate.
Overall score cannot be negative.
Total: 50 pt.

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

**SOLUTION:**

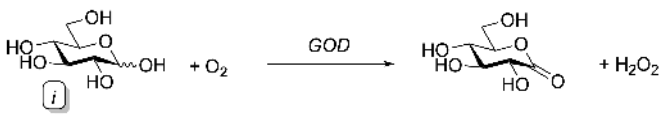
If **Q2.1** is completely correct, in **Q2.2**: 0.4 pt are awarded for each correct identification of substrates i-v.
If **Q2.1** positive reactions are different than the correct answer, but the choice of the substrate in **Q2.2** follows the order of reactivity: 0.4 pt are awarded for each correct identification of substrates i-v.
No points are awarded if the there is more than one possible answer for the substrate based on **Q2.1**.
Overall score cannot be negative.

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

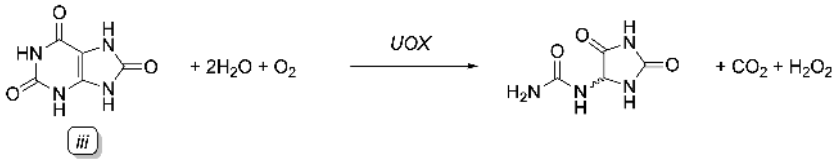
**SOLUTION:**

If **Q2.2** is completely correct, in **Q2.3**: 0.4 pt are awarded for each correct assignment of substrates *i-v* given that there are only two substrates per **S6** - **S10**.
In the case when substrates *i-v* do not match the official solution in **Q2.2**, points are awarded in **Q2.3** based on whether substrates assigned in **Q2.2** would match the reported observations from the reactions between **E1**-**E5** and **S6**-**S10**.
No points are awarded if the there are more than two answers for the substrates based on **Q2.2**.
Overall score cannot be negative.

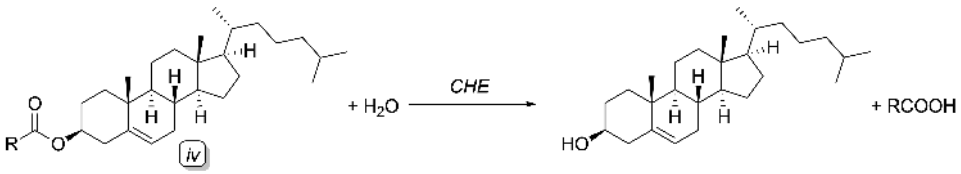
The reaction scheme of enzymes, substrates *i*–*v*, and additional components of mixtures **E1**–**E5** are given below:



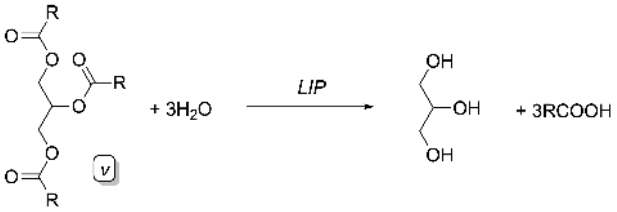




+ 2H2O + O2
UOX
+ CO2 + H2O2

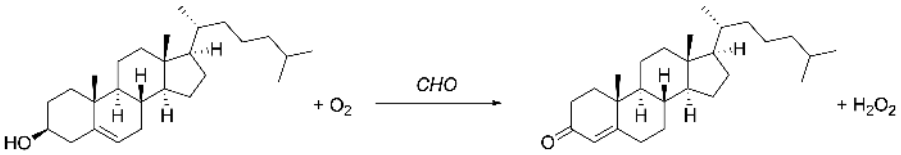
*iii*

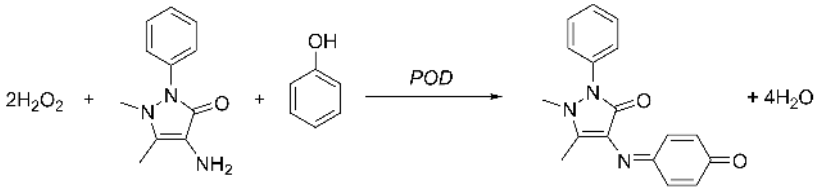

+ H2O
CHE
+ RCOOH

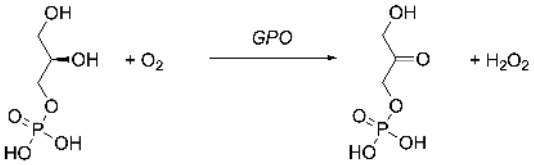
*iv*

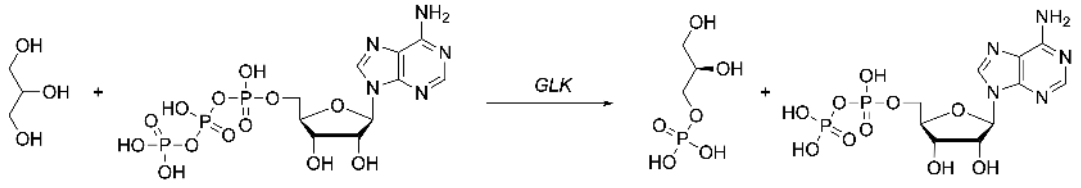

+ 3H2O
LIP
+ 3RCOOH

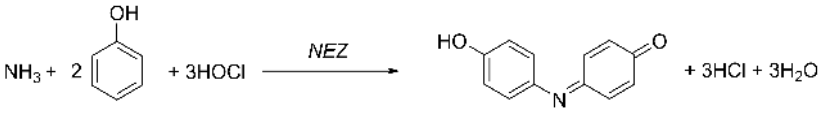
*v*


+ O2
CHO
+ H2O2


2H2O2 +
+
POD
+ 4H2O


+ O2
GPO
+ H2O2


+
GLK
+




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. 1 pt

**Tick** the boxes for the reactions that give coloured products.

**SOLUTION:**

Only two answers are possible according to the task.
For correct answer: 0.5 pt each.
For any 3 or more answers: 0 pt.
Overall score cannot be negative.
According to the task's text, based on the provided reaction scheme for the enzymatic reactions, coloured products originate only from molecules that have conjugated $\pi$-electrons. The only products that satisfy that condition are found in the reactions *POD* and *NEZ*.

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

**SOLUTION:**

| | GOD | URE | UOX | CHE | LIP | CHO | POD | GPO | GLK | NEZ |
|---|---|---|---|---|---|---|---|---|---|---|
| E1-E5 | ☐ | ☐ | ☐ | ☑ | ☐ | ☑ | ☑ | ☐ | ☐ | ☐ |
| | ☐ | ☑ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☑ |
| | ☐ | ☐ | ☐ | ☐ | ☑ | ☐ | ☑ | ☑ | ☑ | ☐ |
| | ☐ | ☐ | ☑ | ☐ | ☐ | ☐ | ☑ | ☐ | ☐ | ☐ |
| | ☑ | ☐ | ☐ | ☐ | ☐ | ☐ | ☑ | ☐ | ☐ | ☐ |

If **Q2.2** is completely correct, then, for **E1**:
For first correct answer: 0.2 pt, For second correct answer: 0.3 pt, For third correct answer: 0.5 pt are summed up.
0 pt for all wrong or more than three correct answers.
For **E3**:
For first correct answers: 0.2 pt, For second correct answer: 0.3 pt, For third and fourth correct answer: 0.5 pt are summed up.
If **Q2.2** is completely correct, then, for **E2**, **E4** and **E5**:
For first correct answer: 0.5 pt, For second correct answer: 0.5 pt are summed up.
0 pt for all wrong or more than two correct answers.
If **E1**-**E5** are assigned different substrates in **Q2.2** than the correct answer, substrates are different than the correct answer, the choice of components in **Q2.5** is graded according to scheme above for those assigned **E1**-**E5**.

## 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

**SOLUTION:**

**F:**

M3 < M1 < M2

**G:**

M2 < M3 < M1

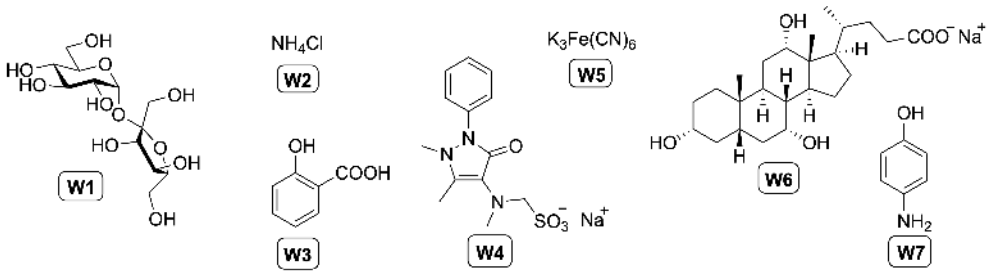
**H:**

M1 < M2 < M3

For each correct relationship between **M1**-**M3** per substrate **F**, **G**, and **H**: 3 pt.
For any wrong answer 0 pt per incorrect answer.
One mistake in any substrate: 0 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

**SOLUTION:**

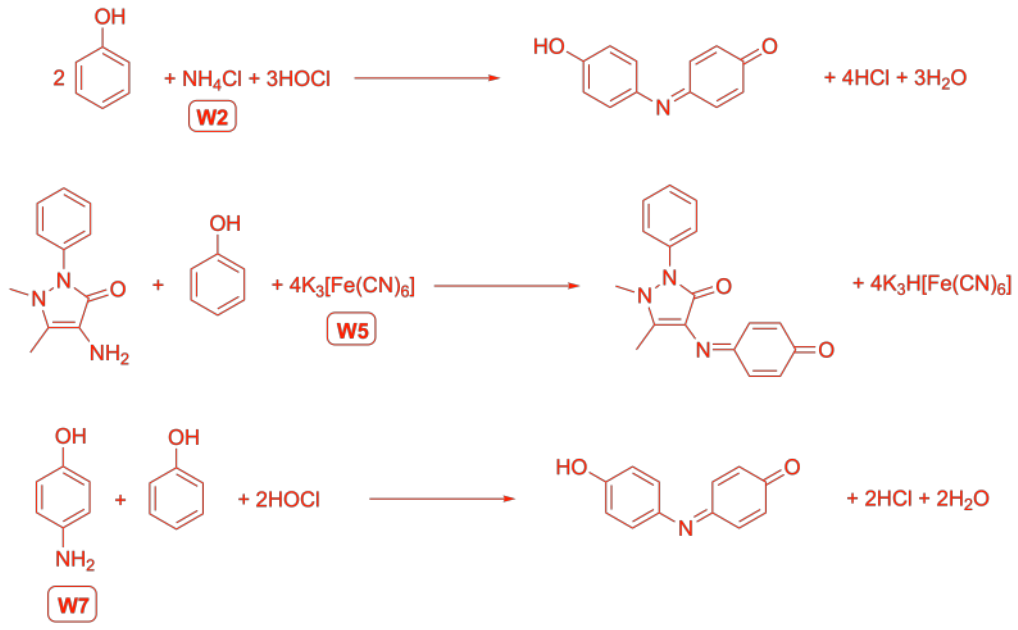
**W1-W7**

| E1-E5 | W1 | W2 | W3 | W4 | W5 | W6 | W7 |
|---|---|---|---|---|---|---|---|
| E1 | ☑ N ☐ Y ☐ G / ☐ B ☐ PR | ☑ N ☐ Y ☐ G / ☐ B ☐ PR | ☑ N ☐ Y ☐ G / ☐ B ☐ PR | ☑ N ☐ Y ☐ G / ☐ B ☐ PR | ☐ N ☐ Y ☐ G / ☐ B ☑ PR | ☑ N ☐ Y ☐ G / ☐ B ☐ PR | ☑ N ☐ Y ☐ G / ☐ B ☐ PR |
| E2a | ☐ N ☑ Y ☐ G / ☐ B ☐ PR | ☐ N ☐ Y ☑ G / ☐ B ☐ PR | ☐ N ☑ Y ☐ G / ☐ B ☐ PR | ☐ N ☑ Y ☐ G / ☐ B ☐ PR | ☐ N ☑ Y ☐ G / ☐ B ☐ PR | ☐ N ☑ Y ☐ G / ☐ B ☐ PR | ☐ N ☐ Y ☑ G / ☐ B ☐ PR |
| E3 | ☑ N ☐ Y ☐ G / ☐ B ☐ PR | ☑ N ☐ Y ☐ G / ☐ B ☐ PR | ☑ N ☐ Y ☐ G / ☐ B ☐ PR | ☑ N ☐ Y ☐ G / ☐ B ☐ PR | ☐ N ☐ Y ☐ G / ☐ B ☑ PR | ☑ N ☐ Y ☐ G / ☐ B ☐ PR | ☑ N ☐ Y ☐ G / ☐ B ☐ PR |
| E4 | ☑ N ☐ Y ☐ G / ☐ B ☐ PR | ☑ N ☐ Y ☐ G / ☐ B ☐ PR | ☑ N ☐ Y ☐ G / ☐ B ☐ PR | ☑ N ☐ Y ☐ G / ☐ B ☐ PR | ☐ N ☐ Y ☐ G / ☐ B ☑ PR | ☑ N ☐ Y ☐ G / ☐ B ☐ PR | ☑ N ☐ Y ☐ G / ☐ B ☐ PR |
| E5 | ☑ N ☐ Y ☐ G / ☐ B ☐ PR | ☑ N ☐ Y ☐ G / ☐ B ☐ PR | ☑ N ☐ Y ☐ G / ☐ B ☐ PR | ☑ N ☐ Y ☐ G / ☐ B ☐ PR | ☐ N ☐ Y ☐ G / ☐ B ☑ PR | ☑ N ☐ Y ☐ G / ☐ B ☐ PR | ☑ N ☐ Y ☐ G / ☐ B ☐ PR |

For substrates **W1**–**W7**:
For each correct choice per enzyme per substrate colour change: 1 pt.
0 pt if there is more than one choice per enzyme per substrate.
Overall score cannot be negative.
Total: 35 pt.

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

**SOLUTION:**




For reaction with **W2**: 0.5 pt.
For reaction with **W5**: 0.75 pt.
For reaction with **W7**: 0.75 pt.
For incorrect stoichiometry: –0.25 points penalty.
Correct balanced equations (either full or ionic) are graded fully.
Overall score for each equation cannot be negative.