**Task 1**

**Answer sheet**

**country: \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**

**team: \_**

**name: signature:**

**name: signature:**

**name: signature:**

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**Hradec Králové**

**Czechia**

**Task A1** (18 points)

**Question A1.1** Draw a representative section from Slide A. Label with terms from Table 1. (7 points)

|  |
| --- |
|  |
| *Do not write anything in this field*Points for slide A: |

**Question A1.2** Draw a representative section from Slide B. Label with terms from Table 1. (7 points)

|  |
| --- |
|  |
| *Do not write anything in this field*Points for slide B: |

**Question A1.3** Enter the letter (A or B) of your slides to the corresponding animal from which the skin sections were prepared. (1 point)

|  |  |
| --- | --- |
| Mouse |  |
| Naked mole-rat |  |

**Question A1.4** Label the epidermal layers in one of your drawings from Question 1.1 or 1.2. (2 points)

**Question A1.5** Which biomolecule is most likely the target of hematoxylin staining? Indicate the corresponding molecule by a tick (√). (1 point)

|  |  |
| --- | --- |
| cellulose |  |
| water |  |
| DNA |  |
| transmembrane proteins |  |
| phospholipids |  |
| simple sugars (oligosaccharides) |  |

**Task A2** (12 points)

**Question A2.1** (8 points)

|  |  |
| --- | --- |
| C1 | C2 |
| D1 | D2 |

**Question A2.2** Write the code of the section (C1, C2, D1, D2) to the corresponding field in the answer sheet. (2 points)

|  |  |  |
| --- | --- | --- |
| **Animal origin** | **Treatment** | **Section code** |
| Mouse | hyaluronidase |  |
| Mouse | mock |  |
| Naked mole-rat | hyaluronidase |  |
| Naked mole-rat | mock |  |

**Question A2.3** Where do the extracellular matrix components come from? Choose the correct statement(s) regarding the protein and polysaccharides molecules found in the matrix and label with by a tick (√) in the answer sheet. (1 point)

|  |  |
| --- | --- |
| They are directly extracted from the surrounding environment. The animal incorporates selected molecules into both superficial and deeper layers of skin. |  |
| They are largely synthetized by skin microbiome (mainly bacteria). Animals with different microbiome display different composition of extracellular matrix. |  |
| They are synthetized solely by liver cells. Blood and lymph transport them to the skin. |  |
| They are synthetized by cells directly in the tissue. Some molecules are made inside the cells and subsequently exported by exocytosis; others are synthetized by transmembrane enzymes. |  |

**Question A2.4** What is the role of HA in skin and other tissues? Pick the correct answer(s) by a tick (√) in the answer sheet. (1 point)

|  |  |
| --- | --- |
| It maintains sufficient hydratation of the tissue. |  |
| It can serve as lubricant, e.g., in joints. |  |
| It has a large space-filling capacity. |  |
| It regulates migration of cells. |  |

**Task A3** (20 points)

**Question A3.1** What type of molecular motors would you expect to be responsible for the transport of melanosomes to the cell periphery? (1 point)

Write the letter of the correct answer in the box

|  |
| --- |
|  |

**Question A3.2** Which absorbance curve corresponds to eumelanins and which to pheomelanins? (0.5 points)

|  |  |
| --- | --- |
| Eumelanins |  |
| Pheomelanins |  |

**Question A3.3** Which statements are correct about eumelanin/pheomelanin absorbtion? Indicate by a tick (√) (select 0–3 answers). (1.5 points)

|  |  |
| --- | --- |
| a) |  |
| b) |  |
| c) |  |

**Question A3.4** Which statements are correct about melanin production? Indicate by a tick (√) (select 0–10 answers). (5 points)

|  |  |
| --- | --- |
| a) |  |
| b) |  |
| c) |  |
| d) |  |
| e) |  |
| f) |  |
| g) |  |
| h) |  |
| i) |  |
| j) |  |

**Question A3.5** In which cases would you expect hyperpigmentation? Choose all true statements and indicate them by a tick (√) (select 0–4 answers). (2 points)

|  |  |
| --- | --- |
| a) |  |
| b) |  |
| c) |  |
| d) |  |

**Question A3.6** Which statements about regulation of sweat production are most likely true? Choose all true statements and indicate them by a tick (√) (select 0–9 answers). (4.5 points)

|  |  |
| --- | --- |
| a) |  |
| b) |  |
| c) |  |
| d) |  |
| e) |  |
| f) |  |
| g) |  |
| h) |  |
| i) |  |

**Question A3.7** Which statements about thermoregulation and regulation of water loss in the naked mole-rat apply? Choose all true statements and indicate them by a tick (√) (select 0–4 answers). (2 points)

|  |  |
| --- | --- |
| a) |  |
| b) |  |
| c) |  |
| d) |  |

**Task B1: Water binding properties**

**Question B1.1** (4 points)



**Graph B1** Total amount of water bind by the hyaluronic acid as a function of time.

|  |  |  |
| --- | --- | --- |
| Time [h:mm] | Diameter [mm] | Weight [g] |
| 0:00 |  | 0.002 |
| 0:15 |  |  |
| 0:30 |  |  |
| 0:45 |  |  |
| 1:00 |  |  |
| 1:30 |  |  |
| 2:00 |  |  |
| 2:30 |  |  |
| 3:00 |  |  |
| 3:30 |  |  |

**Table B1**

**Question B1.2** Draw Graph 2 as the time dependence of the sample diameter, and Graph 3 as the time dependence of weight (12 points)



**Graph B2** Diameter of the sample as a function of time.



**Graph B3** Weight of the sample as a function of time.

**Question B1.3** (4 points)

Is the exponential model good representation of the real water-binding properties of the sample?

**YES NO**

Half-time estimation for sample weight (2 points): \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

**TASK B2: Surface tension of hyaluronic acid**

**Question B2.1.** (8 points)

**Sample 1: Hyaluronic acid**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Number ofmeasurement |  |  |  |  |
| 1 |  |  |  |  |
| 2 |  |  |  |  |
| 3 |  |  |  |  |
| 4 |  |  |  |  |
| 5 |  |  |  |  |
|  |  |  |  |  |
|  |  |  |  |  |
|  |  |  |  |  |

**Sample 2: Water**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Number ofmeasurement |  |  |  |  |
| 1 |  |  |  |  |
| 2 |  |  |  |  |
| 3 |  |  |  |  |
| 4 |  |  |  |  |
| 5 |  |  |  |  |
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**Question B2.2** (1 point)

Decide according to your measurement and circle below the right answer:

a) The surface tension of hyaluronic acid solution is higher than water.

b) The surface tension of hyaluronic acid solution is lower than water.

c) We cannot decide if this is case a) or case b).

**TASK B3: Lubrication properties of hyaluronic acid**

**Question B3.1** (2 points)

Derive the equation and explain what happens in terms of the forces acting on the block (draw picture if necessary):

**Question B3.2** (6 points)

Fill in the tables with measured and calculated values:

**Dry rough side**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Number ofmeasurement |  |  |  |  |
| 1 |  |  |  |  |
| 2 |  |  |  |  |
| 3 |  |  |  |  |
| 4 |  |  |  |  |
| 5 |  |  |  |  |
|  |  |  |  |  |

**Dry smooth side**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Number ofmeasurement |  |  |  |  |
| 1 |  |  |  |  |
| 2 |  |  |  |  |
| 3 |  |  |  |  |
| 4 |  |  |  |  |
| 5 |  |  |  |  |
|  |  |  |  |  |

**Question B3.3** (2 points)

Write down the coefficients of dry friction in for with right number of significant digits:

Dry rough side: \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

Dry smooth side: \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

**Question B3.4** (6 points)

Fill in the tables with measured and calculated values:

**Lubricated rough side**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Number ofmeasurement |  |  |  |  |
| 1 |  |  |  |  |
| 2 |  |  |  |  |
| 3 |  |  |  |  |
| 4 |  |  |  |  |
| 5 |  |  |  |  |
|  |  |  |  |  |

**Lubricated smooth side**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Number ofmeasurement |  |  |  |  |
| 1 |  |  |  |  |
| 2 |  |  |  |  |
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**Question B3.5** (2 points)

Write down the coefficients of dry friction in for with right number of significant digits:

Lubricated rough side: \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

Lubricated smooth side: \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

**Question B3.6** (2 points)

Express as a percentage how much the coefficient of friction has decreased in both cases if the parts have been lubricated with hyaluronic acid.

Rough side: \_\_\_\_\_\_\_\_\_\_\_\_\_ Smooth side: \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

**Task C1**

**Question C1.1** (1.5 points)

How many carbon atoms are in a structural unit of hyaluronic acid?

|  |
| --- |
|  |

Calculate the degree of polymerization of hyaluronic acid chain weighting 150 kDa (kilodalton).

|  |
| --- |
|  |
| *n*(150 kDa polymer): |  |

*Viscosity measurement*

**Question C1.2** Speed of fall of the steel ball (1 point)

|  |  |  |  |
| --- | --- | --- | --- |
| *M* [kDa] | *t* [s] | *l* [mm] | *v* [mm∙s−1] |
| 500 |  |  |  |
| 970 |  |  |  |
| 1610 |  |  |  |
| 1900 |  |  |  |

**Question C1.3** Calculation of viscosity (6.5 points)

|  |
| --- |
|  |

Viscosity of solutions

|  |  |  |
| --- | --- | --- |
| *M* [kDa] | *η* [N∙s∙mm−2] | *η* [Pa∙s] |
| 500 |  |  |
| 970 |  |  |
| 1610 |  |  |
| 1900 |  |  |

**Question C1.4** Empirical coefficients (3 points)

|  |  |
| --- | --- |
| *K* = |  |
| *α* = |  |

*Graph paper for determination of K and α*



*Polymer degradation*

**Question C1.5** Times of ball fall in individual mixtures (5 points)

|  |  |
| --- | --- |
|  | Time of ball fall (measured by the stopwatches) [s] |
| Starting time of individual experiments (according to the wall clock) [hh:mm:ss] | mixture a | mixture b | mixture c | mixture d | mixture e |
|  |  |  |  |  |  |
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**Question C1.6** What is probable mechanism of polymer degradation? Mark the right answer. (2 points)

|  |  |  |
| --- | --- | --- |
| a) electrophilic | b) nucleophilic | c) radical |

Mark the detailed explanation of the reaction mechanism.

a) Electrophilic activation of the molecule of hydrogen peroxide before nucleophile attack of the sugar unit.

b) Catalytic role of the metal ion on peroxide decomposition – formation of HO∙ and HOO∙ radicals.

c) Electrophilic activation of the glycoside bond by coordination to the metal ion before nucleophilic attack of hydrogen peroxide.

d) Electrophilic activation of the glycoside bond by coordination to the metal ion before nucleophilic attack of water molecule.

e) Catalytic splitting of hydrogen peroxide to HO∙ radicals due to intermolecular hydrogen bonds formation with neighbouring hydroxide group after forming a cyclic intermediate.

**Task C2**

**Question C2.1** (1 point)

Value of p*K*A of unsubstituted organic acids is ca 4.8 [e.g. p*K*A(acetic acid) = 4.76, p*K*A(propionic acid) = 4.88, p*K*A(butyric acid) = 4.81].

Mark the reason, why p*K*A of d-glucuronic acid differs from those of mentioned organic acids.

a) Electron withdrawing inductive effect of electronegative oxygen atoms bound to the -carbon.

b) Electron donating inductive effect of free electron pairs of oxygen atoms from hydroxo groups present in the molecule.

c) Carboxylate deprotonation is stabilized by an intramolecular hydrogen bonding due to a presence of neighbouring hydroxo group.

d) Better delocalization of the negative charge in the anion of D-glucuronic acid when compared to mentioned examples.

e) Due to positive mesomeric effect of the hydroxo group bound to the -carbon.

f) Due to a general Pauling-Bell rule of correlation of acidity with number of oxo/hydroxo groups present in the molecule.

**Question C2.2** (1 point)

In what pH region will solution of acetic acid work as the best buffer? Mark the right answer.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| a) pH 0–2 | b) pH 2–4 | c) pH 4–6 | d) pH 6–8 | e) pH 8–10 |

**Question C2.3** (1 point)

In what pH region will lie pH of the 0.1m solution of sodium acetate? Mark the right answer.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| a) pH 2–4 | b) pH 4–6 | c) pH 6–8 | d) pH 8–10 | e) 10–12 |

**Question C2.4**(3 points)

*Factorization of stock solution of NaOH*

Concentration of (CO2H)2

|  |  |
| --- | --- |
| *c*[(CO2H)2] = | mol∙dm−3 |

Consumed volumes of NaOH in factorization

|  |  |  |  |
| --- | --- | --- | --- |
| *V*1 [ml] | *V*2 [ml] | *V*3 [ml] | accepted value of*V* [ml] |
|  |  |  |  |

**Question C2.5** Concentration of stock solution of NaOH (1 point)

|  |
| --- |
|  |
| *c*(NaOH): | mol∙dm−3 |

*Determination of concentration of d-glucuronic acid*

**Question C2.6** Consumed volumes of NaOH in titrations of d-glucuronic acid (3 points)

|  |  |  |  |
| --- | --- | --- | --- |
| *V*1 [ml] | *V*2 [ml] | *V*3 [ml] | accepted value of*V* [ml] |
|  |  |  |  |

**Question C2.7** Concentration of d-glucuronic acid (1 point)

|  |
| --- |
|  |
| *c*(d-glucuronic acid) = | mol∙dm−3 |

*Determination of dissociation constant of d-glucuronic acid*

**Question C2.8** (5 points)

Chosen volume of NaOH

|  |  |
| --- | --- |
| *V*(NaOH) = |  ml |

pH values of prepared solutions – sign of lab assistant needed

|  |  |
| --- | --- |
| pH1 | pH2 |
|  |  |

Determined p*K*A of d-glucuronic acid

|  |  |
| --- | --- |
| p*K*A(d-glucuronic acid) = |  |

**Task C3**

**Question C3.1** (2 points)

What is energy of photon of light used for spectrophotometric measurement (585 nm)? Write the value as frequency (in Hz) as well as wavenumber (in cm−1).

|  |
| --- |
|  |
| *ν*(585 nm) = | Hz | (585 nm) = | cm−1 |

**Question C3.2** (8 points)

Absorbance of prepared solutions – sign of lab assistant needed

|  |  |  |
| --- | --- | --- |
| solution | *c*(*N*-acetyl-d-glucosamine)[mol∙dm−3] | *A*(585 nm) |
| standard 0 | 0 |  |
| standard 1 |  |  |
| standard 2 |  |  |
| standard 3 |  |  |
| standard 4 | 1.00 ∙10-3 |  |
| unknown sample | – |  |

**Question C3.3** (5 points)

|  |  |
| --- | --- |
| *c*(unknown sample): | mol∙dm−3 |

*Graph paper for calibration line*

