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Article title: Assessing what prospective laboratory assistants in biochemistry and cell biology know: Development and validation of the test instrument PROKLAS

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BIOCHEMISTRY (items 1-58)

1 You are supposed to weigh sodium azide. The following pictograms are on the package:

Danger

State where you can weigh the substance.

		Answer option
01	On a laboratory bench	☐
02	On a balance table	☐
03	In a sterile bench	☒
04	Under a fume hood	☐
05	Inside a locked chemical room	☐

2 The Biuret Method is used to determine the concentration of protein in a solution by building up a complex of copper and protein. Indicate as what the resulting liquid waste should be disposed.

		Answer option
01	Halogenated waste	☐
02	Non-halogenated solvents	☐
03	Heavy-metal waste	☒
04	Dilution for the wastewater treatment plant	☐
05	Liquid to be autoclaved	☐

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3 You are using a serum protein electrophoresis
Please check the correct statement.

		Answer option
01	The separation of the proteins is carried out in an acid environment.	☐
02	The proteins are negatively charged in an acidic environment.	☐
03	The separation of the proteins depends, among other things, on their charge, size, and structure.	☒
04	The separation of the proteins is carried out on a sodium azide membrane.	☐
05	The serum protein electrophoresis separates proteins into eight fractions.	☐

4 Check the statement about enzymes which is INCORRECT.

		Answer option
01	Enzymes are so-called biocatalysts.	☐
02	Enzymes form an enzyme-substrate complex.	☐
03	Enzymes exit a reaction without deformation.	☐
04	Enzymes are often substrate-specific.	☐
05	Enzymes belong to the chemical class of lipids.	☒

5 State which of the following statements is INCORRECT.

		Answer option
01	Nephelometry is the measurement of light which falls on a silver-antibody complex and is scattered there.	☐
02	Turbidimetry is the measurement of the cloudiness of a solution in the course of a silver-antibody reaction.	☐
03	Densitometry is the quantification of protein bands after a gel electrophoresis (serum-albumin electrophoresis).	☐
04	During a gel electrophoresis, molecules are separated according to their size.	☐
05	The higher the concentration of the directly measured analyte in a photometry, the higher is the light translucence of the reaction solution.	☒

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6 Mix a buffer solution: c (Tris) = 1.5 mol / L; V = 150 mL; pH = 8.3; M (Tris) = 121 g / mol.

Space for your own calculations.

Evaluate the following statements.

		correct	incorrect
01	You have to weigh 181.5 g Tris.	☐	☒
02	The Tris is dissolved in 150 ml of water.	☐	☒
03	The pH value is adjusted with HCl to c = 1 mol / L.	☐	☒
04	After having adjusted the pH value, you need to top up with water.	☒	☐
05	The pH value is not changed by adding water.	☐	☒

7 State which information is needed to produce 150 mL of 50 mM sodium lye.

		Answer option
01	Molarity of NaOH	☐
02	Concentration of sodium (Na)	☐
03	Amount of hydroxide (OH)	☐
04	Molecular mass of NaOH	☒
05	Density of NaOH	☐

8 State which organic compound is the result of using a Ninhydrin reagent in a thin-layer chromatography.

		Answer option
01	Flavonoids	☐
02	Glucose	☐
03	Amino acids	☒
04	Vitamin C	☐
05	Carbohydrates	☐

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9 In an *Escherichia coli* strain, (safety level S1), the anthrax toxin-producing gene isolated from the anthrax bacillus (safety level S3) is supposed to be cloned. Which laboratory safety level is needed to conduct this work?
Please check the lowest level of security needed.

		Answer option
01	Laboratory without a safety level classification	☐
02	Laboratory compliant with safety level S1	☐
03	Laboratory compliant with safety level S2	☒
04	Laboratory compliant with safety level S3	☐
05	Laboratory compliant with safety level S4	☐

10 A 1% agarose gel is used to analyze DNA fragments. During the evaluation, you notice that the bands are not separated well and are located at the bottom of the gel. You want to produce a new gel with a better separation efficiency. Explain how to proceed.

		Answer option
01	Produce a gel with a lower agarose concentration (e.g. 0.5%)	☐
02	Produce a gel with a higher agarose concentration (e.g. 2%)	☒
03	Shorten the duration of the gel electrophoresis	☐
04	Increase the voltage of the gel electrophoresis	☐
05	An improvement in the separation efficiency is not possible	☐

11 You accidentally drop a sample with the E. coli strain K12 on the laboratory bench; it leaks out. Explain how to proceed because of the potentially hazardous situation.

		Answer option
01	Wipe the table with 30% alcohol	☐
02	Wipe the table with 70% alcohol	☒
03	Wipe the table with 96% waste	☐
04	Wipe table with a surfactant solution	☐
05	Lock the room and arrange a professional waste disposal	☐

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12 The following primers are used in a PCR reaction:
Primer 1: GATGAGTTCGTGTCCGTACAACT
Primer 2: GGTTATCGAAATCAGCCACAGCG.
Calculate the melting temperature of the primers with the Wallace rule.
[Tm = 4 ° C x (G + C) + 2 ° C x (A + T)]

***Space for your own calculations.*)**

		Answer option
01	45 and 49 ° C	☐
02	50 and 52 ° C	☐
03	68 ° C for both	☐
04	68 and 70 ° C	☒
05	70 and 72 ° C	☐

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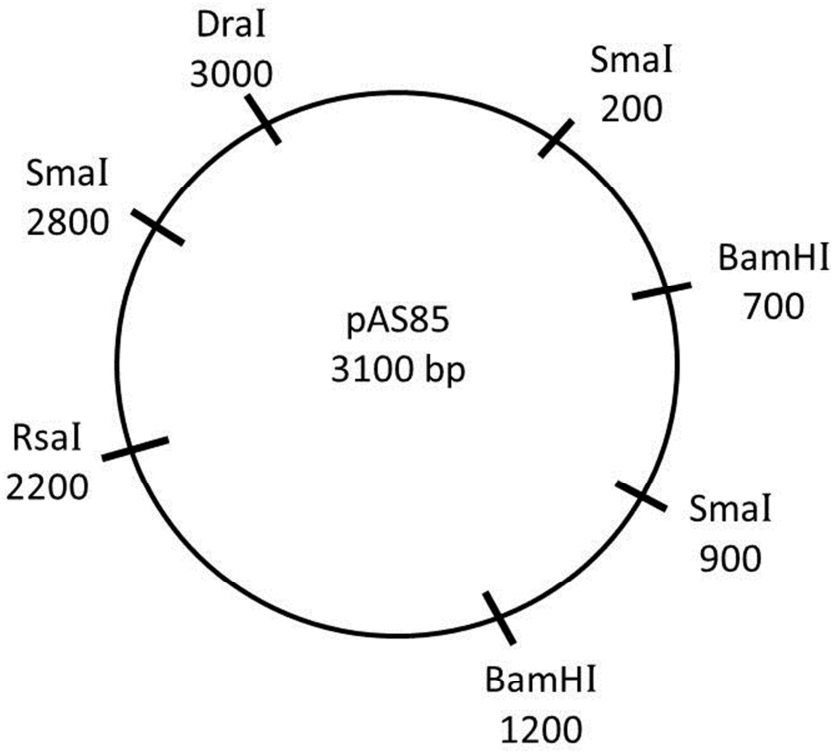
13 The plasmid pAS85 is restricted with the following restriction enzymes. State the size of the resulting fragments.



Please put the results into the number fields, right justified.

restriction enzyme(s)	resulting fragments
*Dra*I	1. 3100
*Rsa*I	1. 3100
*Sma*I	1. 700 2. 1900 3. 500
*Bam*HI	1. 1500 2. 2600
*Dra*I /*Bam*HI	1. 500 2. 1800 3. 800
*Rsa*I / *Sma*I	1. 700 2. 1300 3. 600 4. 500

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14 During an ultracentrifugation run, a sudden imbalance occurs. Determine what to do.

		Answer option
01	Immediately unplug the power cord of the centrifuge.	☐
02	Switch off the centrifuge.	☐
03	Cancel the centrifugation using the stop button.	☒
04	Hold the centrifuge to compensate for the imbalance.	☐
05	Shorten the centrifugation time using the timer.	☐

15 You are preparing a complete documentation in a report. State what kind of information about the label of a protein gel is NOT necessary.

		Answer option
01	Identification of the samples	☐
02	Running conditions	☐
03	Standard sizes	☐
04	Name of the person conducting the experiment	☐
05	cm scale of the gel	☒

16 You are supposed to conduct the purification of a protein from the *Escherichia coli* bacterium. Before cell disruption, you harvest the cells from the culture by centrifugation. State the right treatment to work with the supernatant of the culture.

		Answer option
01	Do nothing. E. coli belongs to risk group 1.	☐
02	Disinfect the supernatant by adding the same volume of ethanol.	☐
03	Autoclave the supernatant.	☒
04	Adjust to pH 9 to avoid the settlement of proteins on the vascular wall.	☐
05	Evaporate the supernatant outdoors.	☐

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17 An ion exchange column is used for the chromatographic separation of proteins. After the sample application, the individual proteins are supposed to be sequentially eluted from the column. For this purpose, you need to change the characteristics of the mobile phase. Indicate how.

		Answer option
01	Change the pH value.	☐
02	Change the flow rate.	☐
03	Change the temperature.	☐
04	Change the ionic strength.	☒
05	Change the pressure.	☐

18 State under which of these conditions you can still safely use an electrical device.

		Answer-option
01	Last electrical test has expired	☐
02	Cable is brittle	☐
03	Housing has cracks	☐
04	Display is defective	☐
05	Housing has scratches	☒

19 State which method is used to determine the antibody titer in blood.

		Answer option
01	ELISA	☒
02	HPLC	☐
03	SDS-PAGE	☐
04	Determination using enzyme tables	☐
05	Mass spectrometry	☐

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20 Which statement with regard to ion-selective electrodes (ISE) is CORRECT?

		Answer option
01	The ISE is used to determine sodium, potassium, and chloride.	☒
02	The ISE is used to determine calcium.	☐
03	The ISE is used to measure protons and neutrons.	☐
04	The ISE is used to easily determine immune complexes.	☐
05	The ISE is used to easily measure lipids.	☐

21 What does the rate of an enzyme-catalyzed reaction depend on?

		correct	incorrect
01	Temperature	☒	☐
02	Substrate concentration	☒	☐
03	External pressure	☐	☒
04	Presence of isoenzymes	☐	☒
05	pH value	☒	☐

22 You need to wear gloves when you stain DNA in agarose gels. Name the reason by checking the correct answer.

		Answer option
01	The colorants leave ugly stains on the hands.	☐
02	The DNA of your own fingers will contaminate the results.	☐
03	The irradiation of UV light will cause thymidine dimers in the human skin.	☐
04	The colorants are partially mutagenic and / or carcinogenic.	☒
05	You always wear gloves when working in the laboratory.	☐

23 Identify the proper disposal method for DNA plasmid waste.

		Answer option
01	It can be disposed together with household waste.	☐
02	It has to be autoclaved before it can be disposed with household waste.	☒
03	It has to be brought to the hazardous waste landfill.	☐
04	It has to be stored in special barrels for half a year before it can be disposed.	☐
05	It must be pasteurized and then disposed by a special disposal company.	☐

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24 You want to produce an antibiotic-containing medium. How do you sterilize this correctly? Check the correct answer.

		Answer option
01	Sterilize the antibiotic-containing medium in a drying cabinet.	☐
02	Autoclave the antibiotic-containing medium.	☐
03	Sterile-filter the antibiotic-containing medium.	☐
04	Autoclave the medium and add the antibiotic after cooling using a sterile filter syringe.	☒
05	Autoclave the medium and antibiotics separately and mix them after cooling.	☐

25 When measuring a DNA solution in a photometer, you receive the error message "first measure blank." State which action you need to take.

		Answer option
01	Verification and correction of parameters	☐
02	Measurement of blank value	☒
03	Measurement of standards to store a valid calibration	☐
04	Dilution of the sample and re-testing	☐
05	Entering a sample number	☐

26 You are cloning plasmid DNA from a safety strain of Escherichia coli K 12 JM109. Explain which measures you need to consider when handling the safety strain in the laboratory.

		Answer option
01	Since this is a safety strain, I do not need to take any precautions.	☐
02	Before and after work cleaning and disinfecting the laboratory is necessary.	☐
03	Remaining bacterial waste can simply be put into the sink.	☐
04	Bacterial waste and contaminated equipment and vessels should be autoclaved or disinfected.	☒
05	The bacterial suspension can be centrifuged in unsealed vessels.	☐

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27 For the preparation of polyacrylamide gels, 100 ml of a gel solution which contains 14.4 g acrylamide (a) and 0.6 g of N, N'-methylenebisacrylamide (b) have been prepared. Calculate the total concentration (T) of acrylamide and N, N'-methylenebisacrylamide, as well as the degree of crosslinking (c) for the acrylamide gel.

$$T = \frac{(a + b) \cdot 100\%}{V}$$

$$C = \frac{b \cdot 100\%}{a + b}$$

		Answer option
01	T = 20% and C = 5%	☐
02	T = 15% and C = 4%	☒
03	T = 15% and C = 5%	☐
04	T = 14% and C = 4%	☐
05	T = 10% and C = 4%	☐

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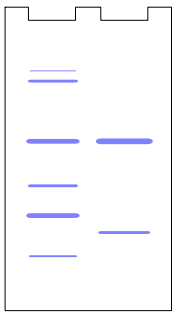
28 Using a discontinuous SDS-polyacrylamide gel electrophoresis, the molar masses of the subunits of Immunoglobulin G (IgG) are to be determined. IgG is composed of four subunits, of which two identical light chains (L chains) and two identical heavy chains (H chains) can be found. Taking into account the migration routes of the protein marker from the pherogram, interpret the molar mass of H-chains and L-chains (in kDa).
Protein Marker (left lane): 118 kDa, 90 kDa, 50 kDa, 36 kDa, 27 kDa, 20 kDa; IgG sample (right lane)



		Answer option
01	36 and 95 kDa	☐
02	25 and 50 kDa	☒
03	50 and 100 kDa	☐
04	25 and 90 kDa	☐
05	10 and 25 kDa	☐

29 The active genes of a liver cell are supposed to be analyzed. For doing so, RNA is isolated, transcribed into cDNA, and then amplified using a PCR. Name two of the following enzymes which are used in this procedure.

		correct	incorrect
01	Reverse transcriptase	☒	☐
02	Primase	☐	☒
03	Human polymerase δ	☐	☒
04	Polymerase I from E. coli	☐	☒
05	Taq polymerase	☒	☐

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30 Negative and positive controls are important when running a PCR. You encounter that the negative control is showing a positive result. Are these statements correct or incorrect?

		correct	incorrect
01	Mix-up of the negative and positive controls	☒	☐
02	Overaging of reagents	☐	☒
03	Primer concentration is too high	☐	☒
04	Inaccurate pipetting and / or opening of the reaction vessels	☐	☒
05	Contamination of reagents	☒	☐

31 State the correct wavelength for measuring an unlabeled protein.

		Answer option
01	260 nm	☐
02	280 nm	☒
03	364 nm	☐
04	405 nm	☐
05	550 nm	☐

32 You have separated proteins in a SDS-polyacrylamide gel according to their size. You have also run a standard size with proteins of known molar mass. How do you determine the molar mass of an unknown protein? Select the correct method.

		Answer option
01	You have to scale the logarithm of the known molecular weights of proteins to their migration distances. In the following, you can calculate the molar mass of the unknown protein on the basis of its migration distance.	☒
02	The migration distance of proteins is not proportional to the molar masses. Therefore, the molar mass of an unknown protein can only be determined when its' migration distance corresponds to one of the standard proteins.	☐
03	The molecular weight of an unknown protein can be calculated using the rule of three approach with the migration distances of the neighboring standard proteins.	☐
04	You cut out the protein band and determine its' dry weight.	☐
05	You compare the color intensity of the unknown protein band with those of standard.	☐

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33 What does Lambert-Beer's law state? Please check the correct answer option.

		Answer option
01	$E = \varepsilon \cdot c \cdot d$	☒
02	$\varepsilon = E \cdot c \cdot d$	☐
03	$c = \frac{\varepsilon \cdot d}{E}$	☐
04	$E = \frac{c \cdot d}{\varepsilon}$	☐
05	$d = \varepsilon \cdot E \cdot c$	☐

34 Name the function of the filter on a photometer.

		Answer option
01	Generation of light	☐
02	Removal of stray light	☐
03	Selection of monochromatic light	☒
04	Prevention of stray light	☐
05	Prevention of fluorescence	☐

35 Explain what is meant by the Michaelis-Menten constant.

		Answer option
01	The affinity of an enzyme for a coenzyme	☐
02	The substrate concentration at which the reaction rate reaches exactly half of the maximum speed	☒
03	The relationship between substrate concentration and enzyme inhibition	☐
04	The relationship between substrate concentration and activators	☐
05	The relationship between substrate concentration and coenzyme	☐

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36 **Polyacrylamide gels are prepared by pouring an acrylamide solution between 2 glass plates and then polymerizing it there. Acrylamide is indicated by the following pictograms:**

Danger

Evaluate the following statements about the handling and disposal of this solution.

		correct	incorrect
01	Wear gloves when pouring the gel.	☒	☐
02	Acrylamide disintegrates in air.	☐	☒
03	The substance is inactivated and harmless when acidified.	☐	☒
04	The solution is disposed of as chemical waste.	☒	☐
05	After polymerization, you can be sure that no acrylamide is present anymore.	☐	☒

37 **You want to destain a SDS-gel. You need ethanol (200 mL), acetic acid (80 mL) and water (520 mL). Where do you find the right H and P phrases and the necessary pictograms for labeling the storage bottle? Evaluate the following statements.**

		Answer-option
01	The instruction on the Material Safety Data Sheet of a renowned manufacturer should be followed.	☒
02	Treat the solution the same way as each of the individual solution it consists of.	☐
03	In such small amounts there is no need to search for the H- and P-phrases.	☐
04	The laboratory security officer is in charge of a risk assessment.	☐
05	The management department should keep a register and files in how to handle such solutions.	☐

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38 Decide whether the following statements regarding the use of protein-containing solutions are correct or incorrect.

		correct	incorrect
01	Enzyme concentrates used for the detection of glucose in food and culture supernatants are maintained at 37 ° prior to use.	☐	☒
02	Frozen samples are mixed well after thawing.	☒	☐
03	Blocking solutions which contain proteins for western blot membranes are usable for three weeks if stored at room temperature.	☐	☒
04	Antibody solutions are stored at 2 - 4 ° C.	☒	☐
05	Frozen enzyme solutions can be thawed when incubated at 95 ° C for 10 min.	☐	☒

39 Antibodies can be efficiently cleaned using a protein A column.

Please check the <u>INCORRECT</u> statement.

		Answer option
01	The antibodies bind with their variable part on the column.	☐
02	The antibodies can be eluted when the pH value of the mobile phase is 2-3.	☒
03	Protein A binds only human antibodies.	☐
04	The column material contains complexed nickel.	☐
05	The elution of the antibodies is carried out by addition of the antigen.	☐

40 Assign the following statements to macro enzymes.

		correct	incorrect
01	A complex of enzyme with albumin.	☐	☒
02	A complex of enzyme and immunoglobulins.	☒	☐
03	An oligomer of enzymes.	☒	☐
04	An increased concentration of coenzymes.	☐	☒
05	An increased concentration of activators.	☐	☒

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41 Where do you NOT get information on the storage of reagents? Please state by checking.

		Answer option
01	Internet	☐
02	Material Safety Data Sheet	☐
03	Merck Index	☐
04	Laboratory Safety Officer	☐
05	Management	☒

42 You want to determine the concentration of your prepared DNA by using a photometer. Name the wavelength at which DNA has its' absorption maximum.

		Answer option
01	340 nm	☐
02	180 nm	☐
03	260 nm	☒
04	640 nm	☐
05	420 nm	☐

43 Water is sometimes treated with DEPC. State the reason.

		Answer option
01	To make the water free of lipase	☐
02	To make the water free of protease	☐
03	To make the water free of amylase	☐
04	To make the water free of RNase	☒
05	To make the water free of cellulase	☐

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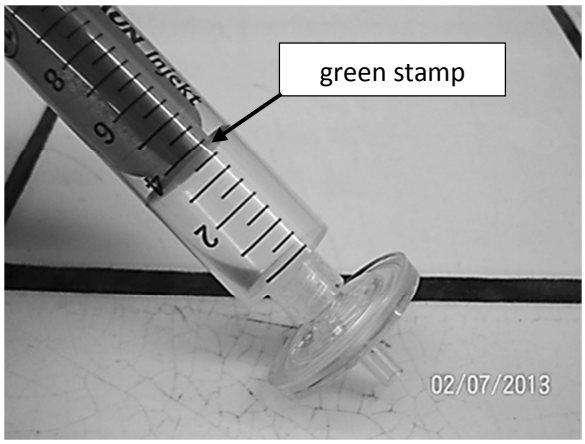
44 State the proper procedures for sterile filtration of small amounts of samples using a sterile filter and a syringe.



		Answer option
01	The sterile filter is placed on the bottom, the green stamp is removed, the sample is loaded, the green stamp is put in again, and the sample is injected into a sterile vessel through the sterile filter.	☐
02	The sample is drawn up into the syringe without the filter and then injected into a sterile vessel through the sterile filter.	☒
03	The sample is drawn up into the sterile syringe through the sterile filter. Then the sterile filter is removed and the sample is placed in a sterile vessel.	☐
04	The sample is drawn up into the sterile syringe through the sterile filter and then passed through the sterile filter back into a sterile vessel.	☐
05	The sample is drawn up into the sterile syringe through the sterile filter, the green stamp is removed, and the sample is placed in a sterile vessel.	☐

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45 You isolate DNA from leukocytes and measure the optical density to determine the DNA concentration. This measurement reveals a 1.2 absorption ratio of the A260 nm and A280 nm wavelengths. What does this value tell you about the purity of your prepared DNA? State the correct answer.

		Answer option
01	The solution contains DNA.	☐
02	The solution contains DNA and proteins.	☒
03	The solution contains DNA and cell-wall components.	☐
04	The solution contains bacteria.	☐
05	The solution contains RNA.	☐

46 What does EDTA cause in a buffer used for DNA isolation? State the correct and incorrect effects.

		correct	incorrect
01	EDTA inhibits Mg2+ ions which are necessary to maintain the structure of the cell wall.	☒	☐
02	EDTA inhibits DNA-degrading enzymes.	☒	☐
03	EDTA promotes the solubility of lipids in the cell membrane.	☐	☒
04	EDTA forms insoluble RNA complexes.	☐	☒
05	EDTA is used to precipitate proteins.	☐	☒

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47 You want to harvest bacteria for a transformation. The optimal growth phase is reached when the bacterial suspension shows an optical density (OD_{600nm}) of 0.35. You need a total of 5 x 10^{10} / mL of bacteria. Calculate which amount of bacterial suspension you have to produce.

1 OD_{600nm} = 8 x 10^8 bacteria / mL

Space for your own additional calculations.

		Answer option
01	about 70 mL	☐
02	about 130 mL	☐
03	about 180 mL	☒
04	about 240 mL	☐
05	about 350 mL	☐

48 Which restriction enzyme is needed to cut open a plasmid when it contains the sequence 5'-ACCTGCAGATT-3'? Please check the correct answer.

			Answer-option
01	*ApaL*I	with the cutting sequence 5'-GTGCAC-3'	☐
02	*BamH*I	with the cutting sequence 5'-GGATCC-3'	☐
03	*EcoR*I	with the cutting sequence 5'-GAATTC-3'	☐
04	*Hind*III	with the cutting sequence 5'-AAGCTT-3'	☐
05	*Pst*I	with the cutting sequence 5'-CTGCAG-3'	☒

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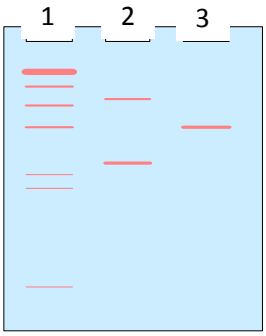
49 **After an electrophoretic separation, plasmid pBR322 (bp 4361) DNA loaded onto agarose gel shows two bands in a pherogram. When the plasmid is cut with the restriction enzyme Eco RI, the gel only shows one band, which is arranged between the two bands of the uncut DNA. Explain the different migration routes of cut and uncut plasmid DNA.**

DNA marker (lane 1): 23130bp, 9416 bp, 6557 bp, 4361 bp, 2322 bp, 2027 bp, 564 bp; uncut plasmid DNA (lane 2); cut with Eco RI plasmid DNA (lane 3)



		correct	incorrect
01	The uncut plasmid DNA contains the open ring and the supercoiled form.	☒	☐
02	The isolated plasmid is heavily contaminated with foreign DNA.	☐	☒
03	The cut plasmid DNA is present in a linear double-stranded form and travels at the same speed as a corresponding DNA fragment in the DNA markers.	☒	☐
04	The supercoiled form of plasmid DNA is very compact and therefore travels quickly through the gel.	☒	☐
05	In gel electrophoresis, the spatial structure of the DNA is not important.	☐	☒

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50 Using an agarose gel electrophoresis, DNA fragments of the sizes 500-6000 bp are supposed to be separated. Provide the required amount of agarose (w / v) for the electrophoresis gel.

		Answer option
01	0.2%	☐
02	0.5%	☐
03	1.2%	☒
04	1.8%	☐
05	2.0%	☐

51 When a PCR program is finished, the PCR reactions are cooled in the thermal cycler at 4 ° C. State the main reason for this cooling step.

		Answer option
01	Excess dNTPs in the PCR solution would otherwise eliminate their phosphate residues.	☐
02	Without the cooling step, the DNA polymerase would catalyze unspecific primer extensions.	☒
03	Without the cooling step, the DNA polymerase would decompose.	☐
04	The PCR products would be reduced at higher temperatures.	☐
05	Actually, the cooling step is unnecessary.	☐

52 In a PCR approach with a total volume of 50 µL, the concentration of Mg^{2+} ions should be 2 mmol / L. As a stock solution, an MgCl2 solution with a molar concentration of 25 mmol / L is available. Evaluate which volume of the $MgCl_2$ solution must be pipetted into the PCR mixture.

Space for your own calculations.

		Answer option
01	5 µL	☐
02	10 µL	☐
03	4 µL	☒
04	3 µL	☐
05	2 µlL	☐

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53 For the implementation of gel electrophoreses, 250 mL of a 10-fold TBE buffer are available. Calculate the maximum volume of 1x TBE buffer can be prepared from the 10x buffer?

Space for your own calculations.

		Answer option
01	0.5 L	☐
02	2.5 L	☒
03	1.0 L	☐
04	2.0 L	☐
05	5.0 L	☐

54 State the aim which is pursued in carrying out a PCR.

		Answer option
01	Replication of the entire DNA.	☐
02	Amplification of a defined portion of a DNA.	☒
03	Reproduction of the primer.	☐
04	Cutting the DNA into shorter fragments.	☐
05	Determination of the nucleotide sequence in the primers.	☐

55 You want to centrifuge equally filled test tubes in a centrifuge with a six-place rotor. Check all options which specify a correct number of test tubes which can be centrifuged simultaneously.

		correct	incorrect
01	2	☒	☐
02	3	☒	☐
03	4	☒	☐
04	5	☐	☒
05	6	☒	☐

56 State the correct structure of human DNA.

		correct	incorrect
01	single-stranded	☐	☒
02	double-stranded	☒	☐
03	linear	☒	☐
04	circular	☐	☒
05	ssDNA	☐	☒

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57 Many plasmids contain a polylinker (multiple cloning site = MCS). State the reason.

		Answer option
01	It is necessary in order to start the replication of the plasmid at the origin.	☐
02	It is necessary in order to not destroy the plasmid when cloning.	☐
03	It is important in order to insert the DNA into the plasmid easily when cloning.	☒
04	It is necessary in order to link restriction enzymes.	☐
05	It is necessary in order to stop the translation of the plasmid.	☐

58 It is necessary to follow the rules of Genetic Engineering Act when working in genetic engineering. Determine whether the rules have to be followed during these operations.

		Answer option
01	When culturing the laboratory strain of E. coli JM109 without plasmids.	☐
02	When culturing the laboratory strain of E. coli JM 109 with a plasmid insert (pZL1).	☒
03	When restricting the plasmids with inserts (pLZ1).	☐
04	When conducting a PCR of the gene region PV92 in the human genome.	☐
05	When transcribing RNA of the gene region PV92 in the human genome.	☐

CELL BIOLOGY (items 59-92)

59 A great number of plants contain substances of which tiny doses can already disturb the metabolism of living organisms and can directly or indirectly exert toxic effects. What do you need to consider while cultivating and processing plants which contain hazardous substances?

		Answer option
01	Sufficiently protect plants against environmental influences.	☐
02	Protect other plants from the ingredients.	☐
03	Do not bring plants into contact with living organisms.	☐
04	Protect people with personal protective equipment.	☒
05	Ingredients should remain in the plants if possible.	☐

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60 You are measuring the oxygen production of isolated chloroplasts. For doing that, you degenerate the electron transport with dinitrophenyl (DNP), so that proton gradients are destroyed and the energy flow inside the cells of living organisms is stopped. State how to deal with a small amount of DNP waste.

		Answer option
01	Pour the waste down the laboratory sink or directly dispose into the public garbage can.	☐
02	Identify waste authority or immediately notify the local garbage disposal service.	☐
03	Heat waste slightly in a double boiler and evaporate appropriately under hood.	☐
04	Collect waste according to regulations and relevant national regulations for a proper disposal.	☒
05	Freeze waste quickly and permanently stored in freezer at -18 ° C.	☐

61 Specify what you need to consider when autoclaving liquids.

		Answer option
01	Vessels should always be tightly closed.	☐
02	Only one vessel at a time should be autoclaved.	☐
03	Fluids must not be autoclaved.	☐
04	Vessels should be slightly open.	☒
05	Liquid must be mixed well before autoclaving.	☐

62 You have received two petri dishes which contain merely a culture medium from your lab manager. You are supposed to place them openly into a sterilized safety workbench. Explain the reason for such a work order.

		Answer option
01	The laminar air flow needs to be pointed out.	☐
02	The culture media need to be sterilized.	☐
03	It is used to check your knowledge because this is not allowed.	☐
04	The sterilized safety workbench is supposed to be contaminated to show you the relevance of working under sterile conditions.	☐
05	It is used to check whether the safety workbench was sufficiently sterilized.	☒

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63 Point out what the term "sterile" stands for.

		Answer option
01	Not capable of reproduction	☐
02	Free of bacterial DNA	☐
03	Heated	☐
04	Nonviable	☒
05	Free of media	☐

64 Name under which conditions culture media are autoclaved.

		Answer option
01	100 ° C for 20 min	☐
02	121 ° C for 20 min	☒
03	85 ° C for 10 min	☐
04	135 ° C for 3 seconds	☐
05	72 ° C for 15 seconds	☐

65 Explain what is meant by the term "pasteurization."

		Answer option
01	A complete sterilization.	☐
02	A partial disinfection after which only pathogens survive.	☐
03	A partial disinfection during which only pathogens are killed.	☒
04	A partial disinfection during which all saprophytes are killed.	☐
05	A neutralization of food taste.	☐

66 Agar agar is a polysaccharide from algae and is used as nutrition for culture media. State the purpose of such an additive.

		Answer option
01	nitrogen source	☐
02	carbohydrate source	☐
03	buffering agent	☐
04	gelling agent	☒
05	lubricant	☐

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67 State the approach to cultivate an aerobic culture.

		Answer option
01	Incubation in high layer	☐
02	Shaking culture	☒
03	Koch's method to pour media plates	☐
04	Incubation using an anaerobic jar	☐
05	Incubation in low layer	☐

68 Which object lens with a 10x eyepiece magnification is needed for a precise light microscopic examination of bacteria? Please check the correct answer.

		Answer option
01	2.5x object lense	☐
02	4x object lense	☐
03	10x object lense	☐
04	40x object lense	☐
05	100x object lens (oil immersion object lense)	☒

69 What do you need to know prior to the disposal of laboratory waste? Please check the correct answer.

		Answer option
01	Chemical composition of the material	☒
02	Temperature of the material	☐
03	pH value of the material	☐
04	Color of the material	☐
05	Smell of the material	☐

70 Which of the following is the prescribed method for inactivating liquid and solid waste? Please check the correct answer.

		Answer option
01	flaming	☐
02	disinfection	☐
03	sterile filtration	☐
04	UV radiation	☐
05	autoclaving	☒

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71 Which of the following could compromise sterility during sterile work? Please check the correct answer.

		Answer option
01	Touching the work surface with the pipette tip.	☒
02	Flaming glass bottles.	☐
03	Holding bottles in a 45-degree angle while opening.	☐
04	Open bottles only as long as necessary.	☐
05	Do not put down the lid.	☐

72 Disposable materials (like pipettes or vessels) are often used when working with cell cultures. State how to correctly dispose such material.

		Answer option
01	Autoclave and then dispose as residual waste.	☒
02	Dispose directly as residual waste.	☐
03	Autoclave and dispose as hazardous waste.	☐
04	Dispose in the can for recycling packages and plastics.	☐
05	Dispose as hazardous waste.	☐

73 The abbreviation GLP stands for Good Laboratory Practice. Arrange the correct statement to the corresponding concept.

		Answer-option
01	Quality assurance system for laboratory databases	☐
02	Quality assurance system for clinical safety tests	☐
03	Quality assurance system for health safety studies	☒
04	Quality assurance system for the training of laboratory technicians	☐
05	Quality assurance system for the production of genetic cloning	☐

74 Incubatorsfor cell cultures are gassed with CO2. State which safety regulations must be followed when a compressed gas cylinder is stored in the laboratory.

		correct	incorrect
01	Gas cylinders must be stored in secure cooling chambers.	☐	☒
02	Gas cylinders must be secured against falling over with chains.	☒	☐
03	Gas cylinders must be stored horizontally and protected against rolling.	☐	☒
04	Valves must be protected against tearing with protective caps.	☒	☐
05	No special safety regulations must be followed for non-flammable gases.	☐	☒

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75 When cultivating cells, safety measures must be taken. Assign the correct safety measures to the areas of protection.

		Protecting cell cultivation	Protecting people
01	Doors must be kept closed during the procedure.	☒	☐
02	Eating or drinking is not allowed in the laboratories.	☒	☐
03	After the procedure is finished, wash and disinfect your hands.	☐	☒
04	Avoid aerosols.	☒	☐
05	Only the needed equipment should be placed on the laboratory table .	☒	☐

76 Assess the risks which can originate from an established cell line of risk group 1.

		correct	incorrect
01	Cell cultures may contain retroviral DNA sequences.	☒	☐
02	People without antibodies against hepatitis B may work with these cells.	☒	☐
03	Nursing mothers are allowed to work with these cultures.	☒	☐
04	There are no known incidents of any threat to humans or the environment.	☒	☐
05	Working with these cell lines is permitted only in safety workbenches.	☐	☒

77 State the substances which are <u>NOT</u> used for the preparation and coloration of animal tissues and pose a health hazard.

		Answer option
01	Formalin	☐
02	Hematoxylin	☒
03	Eosin	☐
04	Cyanin	☐
05	Methanol	☐

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78 State the components of a microscope which are needed for a phase contrast microscopy.

		Answer option
01	UV lamp	☐
02	Tubus	☒
03	Oil immersion lens	☐
04	Condenser with aperture diaphragm	☐
05	Condenser without aperture diaphragm	☐

79 You have extracted the photosynthesis pigments from a tobacco plant using acetone (F, Xi). You subsequently separated them by a thin layer chromatography using a DC plastic film. After the frontline has moved 8 cm away from the starting point, you stop the chromatography. Then, you measure that the green-colored lines of your samples went a distance of 6.2 cm from the start. You calculate the retention factor (Rf) by dividing the measured distance of the track start line to the line of the substance Sx by the track start line to the solvent front Sf:

$$R_f = \frac{S_x}{S_f}$$

On the basis of the value R_f, determine which photosynthesis pigment can be assigned to the line.

Space for your own calculations.

		Answer option
01	Carotene ($R_f = 0.98$)	☐
02	Chlorophyll a ($R_f = 0.82$)	☐
03	Chlorophyll b ($R_f = 0.78$)	☒
04	Lutein ($Rf = 0.74$)	☐
05	Neoxanthin ($R_f = 0.54$)	☐

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80 You want to autoclave some bottles with media. State which temperature you should set the autoclave at.

		Answer option
01	100°C	☐
02	121°C	☒
03	180°C	☐
04	200°C	☐
05	225°C	☐

81 State for which findings no further diagnostics are required.

		Answer option
01	Midstream urine: bacterial count 10^5 CFU / mL, inhibitors not detected	☐
02	Midstream urine: bacteria count 10^5 detected CFU / mL, inhibitors	☒
03	Bladder puncture urine: bacterial count 10^5 CFU / mL, inhibitors not detected	☐
04	Midstream urine: bacteria count: 10^2 cfu / mL, inhibitors not detected	☐
05	Bladder puncture urine: bacterial count 10^2 CFU / mL, inhibitors not detected	☐

82 You want to collect information in order to examine ticks for *Borrelia burgdorferi*. You believe it would be the best to browse for articles in journals and the internet. State the web page which gives you access to suitable articles.

		Answer option
01	NIB Tickmed (National Institute of Biology)	☐
02	NCBI Pubmed (National Center for Biotechnology Information)	☒
03	NOW Pubbac (National Office for Wildlife)	☐
04	NFA DNA Bank (National Forest Administration)	☐
05	HortiPlex database (Horticultural Database)	☐

83 State which of the following statements describes the principle of Neubauer's counting chamber to determine the cell number of a cell suspension.

		Answer option
01	Inoculation with nutrient solution after dilution	☐
02	Cells are counted after incubation	☐
03	Cells are cultivated in Neubauer's counting chamber	☐
04	Cell count in the visible quadrant suggests the total number of cells	☒
05	Sample is diluted in decimals	☐

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84 The enzyme RubisCO (ribulose-1.5-bisphosphate carboxylase) fixes CO_2 as a key enzyme of the Calvin cycle in the so-called dark reaction of photosynthesis. You want to purify the enzymes of the Calvin cycle from isolated chloroplasts. State where in a chloroplast the RubisCO can be localized.

		Answer option
01	In the outer membrane of a chloroplast	☐
02	In the thylakoid membrane of a chloroplast	☐
03	In the matrix (stroma) of a chloroplast	☒
04	Inside a thylakoid granule of a chloroplast	☐
05	In the inner membrane of a chloroplast	☐

85 A first important distinction for the determination of bacteria is the classification into Gram-positive and Gram-negative. This distinction can be easily realized with the so-called Gram staining. Arrange the correct steps for a Gram staining in a chronological order.

		Answer option
01	Heat fixation, forming a water-soluble paint (Lugol's solution), crystal violet staining, differentiation (wash color out with ethanol), counterstaining with safranine	☐
02	Crystal violet staining, heat fixation, counterstaining with safranine, differentiation (wash color out with ethanol), formation of a water-soluble paint (Lugol's solution)	☐
03	Heat fixation, crystal violet staining, formation of a water-soluble paint (Lugol's solution), differentiation (wash color out with ethanol), counterstaining with safranine	☒
04	Heat fixation, differentiation (wash color out with ethanol), crystal violet staining, formation of a water-soluble paint (Lugol's solution), counterstaining with safranine	☐
05	Crystal violet staining, formation of a water-soluble paint (Lugol's solution), heat fixation, counterstaining with safranine	☐

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86 Lugol's solution is used for Gram staining. State the role of iodine in this process.

		Answer option
01	Iodine colors the bacterial cell wall blue due to its high starch content.	☐
02	Together with crystal violet, iodine forms an iodine dye complex which cannot be washed out.	☒
03	Iodine facilitates the penetration of the crystal violet solution into the cell.	☐
04	Iodine kills pathogenic organisms.	☐
05	Iodine helps the dye attach to the cytoplasmic membrane.	☐

87 To produce a selective nutrition medium for a bacterial cultivation, an antibiotic is needed. State the correct procedure.

		Answer option
01	The antibiotic is autoclaved together with the remaining ingredients of the cultivation medium.	☐
02	The antibiotic is isolated from the cultivation medium, autoclaved, and then added to the nutrition medium under sterile conditions.	☐
03	The antibiotic is sterile-filtered and added to the autoclaved cultivation medium when the medium is cooled down.	☒
04	In a final step, the antibiotic is added on top of already prepared media plates.	☐
05	The antibiotic is added to the autoclaved cultivation medium immediately after sterilization.	☐

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88 For a more precise evaluation of bacteria, preparations are stained. Which of the options is the most important polychromatic coloring? Please check the correct answer.

		Answer option
01	Neisser staining	☐
02	Ziehl-Neelsen staining	☐
03	Differential staining according to GRAM	☒
04	Spore staining according to DORNER	☐
05	Methylene blue solution according to LÖFFLER	☐

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89 Why is a heat fixation performed prior to the Gram staining? Please check the correct answer.

		Answer option
01	The colors appear more brilliant after the heat fixation.	☐
02	The bacteria do not become deformed by the heat fixation.	☐
03	After the heat fixation, the bacteria can be distinguished into cocci and rods.	☐
04	After the heat fixation, the bacteria can be distinguished into gram-negative and gram-positive bacteria.	☐
05	The preparation no longer detaches from the slide by protein denaturation.	☒

90 Which urine can be used for a bacterial count measurement in the case of an acute urinary tract infection? Please check the correct answer.

		Answer option
01	Midstream urine	☒
02	24-hour urine collection	☐
03	Urine after a 24-hour incubation at 37°C	☐
04	Urine after cold enrichment	☐
05	Urine after fixation	☐

91 Tissue cell cultures are grown in special cultivation flasks. State under which conditions adherent cells can be grown.

		correct	incorrect
01	Bottles must be sealed airtight.	☐	☒
02	Bottles must be sterile.	☐	☒
03	Bottles must be large.	☒	☐
04	Bottles should be of good optical quality.	☐	☒
05	Bottles should consist of cloudy glass.	☐	☒

92 Cell lines can be stored in liquid nitrogen at – 196 ° C for years without losing their viability. Assess the following statements.

		correct	incorrect
01	Cell lines can be also stored at - 20 ° C.	☐	☒
02	Cell lines can be also stored at - 80 ° C.	☒	☐
03	Glycerin is used as a protective substance.	☒	☐
04	Dimethylsulfoxide prevents the crystal formation of water inside and outside of the cell.	☒	☐
05	The freezing process should be slow.	☒	☐