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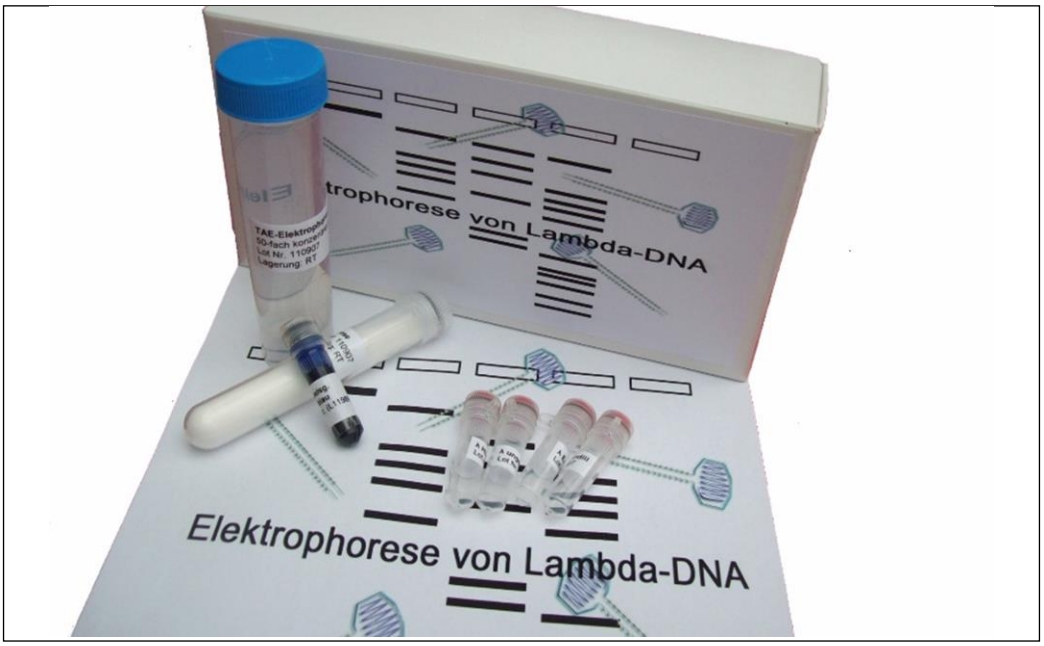
Operating manual



[Kit: Agarose gel electrophoresis of lambda DNA] [KLA-530-110]

I. Area of application/introductory remark

These instructions describe the preparation of lambda DNA in agarose gel electrophoresis. During the experiment, undigested (uncut) lambda DNA will be compared to lambda DNA that has been digested (cut) by way of enzymes.

The basis of the lambda DNA kit is the double-stranded DNA genome with 48,502 base pairs of the lambda bacteriophage.

Details on the lambda phage can be found in specialist literature or Internet databases.

II. Content

Lambda DNA	120 µl
Lambda DNA, Eco RI, cut	120 µl
Lambda DNA, Hind III, cut	120 µl
Lambda DNA, Eco RI / Hind III, cut	120 µl
Agarose	6 g
TAE electrophoresis buffer (50x concentration)	50 ml
DNA staining solution (200x concentration)	1.5 ml

Note: The DNA samples are ready for use and can be directly used for the gel electrophoresis. If the DNA samples need to be stored for a longer period of time, they should be deep-frozen at -18°C.
The DNA samples include the dye bromophenol blue (BPB). This dye is used to monitor the progress of the gel electrophoresis. After the gel electrophoresis, the DNA fragments are stained with the aid of the supplied DNA staining solution.

III. Preparation of the experiment

Electrophoresis buffer

Dilute the electrophoresis buffer (50x concentration) (or part of it) with distilled water until a 1x concentration is reached. This electrophoresis buffer (1x concentration) will then be used for the experiment. It can be reused.

Casting the agarose gel

We recommend using a 1.2% agarose gel for the electrophoresis of the DNA fragments. Depending on the electrophoresis chamber that is used, different gel volumes are required. The quantities are stated in the handbook of the electrophoresis chamber.
The same applies to the correct utilisation of the electrophoresis chamber. Many electrophoresis chambers permit the gel to be cast 1-2 days before the actual experiment. This can be useful for reasons of time and organisation.

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Tip: In order to prepare the agarose gel, weigh the required quantity of agarose, fill it into an Erlenmeyer flask, add a corresponding volume of the electrophoresis buffer and seal the flask slightly by way of a cotton-wool stopper. Prior to heating the material in the microwave oven, note down the total weight of the Erlenmeyer flask (flask including the content) so that - after the dissolution of the agarose solution - the loss due to boiling can be compensated for with distilled water. This ensures that the desired percentage of the agarose gel is reached. The gel should not be cast too thick, since this would have a negative effect on the staining of the DNA fragments after the completion of the gel electrophoresis. Gel with a thickness of 3 to 4 mm is ideal for this experiment.

Preparation of the DNA staining solution
Dilute the staining solution (200x concentration) with distilled water so that a staining solution with 1x concentration results. This means that 1 volume of the staining solution with 200x concentration must be added to 199 volumes of distilled water in order to produce the ready-for-use staining solution. Store in a dark place at 4°C in a refrigerator. The DNA staining solution can be reused.

Separation of the cohesive ends
The lambda phage has ends that consist of single-stranded DNA. Since they are complementary to one another, they can either form circular structures or several lambda DNA molecules form a sort of chain (concatemers).
In order to avoid artefacts during gel electrophoresis, the cohesive ends (also known as sticky ends) of the lambda DNA (all 4 samples) should be separated prior to the gel electrophoresis. To this end, the DNA samples are heated at 65°C for 5 minutes and then immediately cooled on ice (on crushed ice or placed in the freezer compartment in a pre-cooled vessel rack with good contact with the vessels).

IV. Procedure

Tip: Experience has shown that pipetting with a microlitre pipette can be difficult for beginners. This is why the pipetting process and the filling of the samples into the gel pockets of the agarose gel should be trained prior to the experiment. A suitable training solution is a solution of blue ink (2 volumes) and glycerine (1 volume). This training solution has approximately the same viscosity as the supplied DNA samples which makes it particularly suitable for training purposes.

Common problems: It can be difficult to hit the gel pockets precisely and sometimes the pockets are overfilled. In other cases, the tip of the pipette may be inserted into the gel pocket too deep so that the bottom of the gel pocket is damaged. Another common problem is that the tip of the pipette is withdrawn from the gel pocket too quickly so that part of the sample, which has just been dispensed into the pocket, will also be dragged out of the pocket. The pushbutton of the micropipette does not remain pushed down when the tip of the pipette is withdrawn so that the sample will be drawn back into the pipette.

Electrophoresis of the DNA samples
Transfer 12 µl of each of the DNA samples into the gel pockets of the agarose gel by way of a microlitre pipette in the order that is stated below:

- Lambda DNA, uncut
- Lambda DNA, Eco RI
- Lambda DNA, Hind III
- Lambda DNA, Eco RI / Hind III

Ensure that the bottom of the gel pockets is not damaged, i.e. do not insert the tip of the pipette too deep into the gel pockets. Start the electrophoresis immediately after the application of the DNA samples. The direct voltage that is to be adjusted depends on the electrophoresis chamber that is used. As a rule of thumb, a voltage of 5 Volts/cm (distance between electrodes) should be used. Stop the electrophoresis when the dye bromophenol blue in the DNA samples has reached the lower edge of the agarose gel.

Staining of the DNA bands
After the electrophoresis, transfer the agarose gel carefully into a suitable staining dish. The staining dish can be made of plastic or glass. Use a commercially available kitchen spatula or a wide putty knife, for example, for transferring the gel.
Overlay the gel with the staining solution and stain the gel for approximately 10 to 15 minutes. Shake the dish slightly during this process in order to ensure homogeneous staining. Then, pour the staining solution back into the storage bottle and destain the gel with tap water until the background has been sufficiently destained so that the DNA bands are visible. The gel should then ideally be photographed with transmitted light on a light box. The gel can be wrapped into cling film and stored in a refrigerator for a few days. Following overnight storage in a refrigerator, the DNA bands are often more visible with a higher contrast than the day before.

V. Evaluation

Following the separation of the DNA samples under optimum electrophoresis conditions and optimum staining of the DNA, the following fragment lengths can be observed (values stated in base pairs, bp):

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Lambda DNA - undigested	Lambda DNA - EcoR1	Lambda DNA - HindIII	Lambda DNA EcoR1/HindIII
48.502*	21.226*	23.130*	21.226*
	7.421	9.416	5.148
	5.804	6.557	4.973
	5.643	4.361	4.268
	4.878	2.322	3.530
	3.530	2.027	2.027
		564	1.904
		125	1.584
			1.375
			947
			831
			564
			125

*: DNA fragments with cohesive ends

Small DNA fragments are often not visible, since the supplied dye that is used for staining is not sensitive enough in order to stain relatively short DNA fragments.
If the migration distance is too short, it may be impossible to separate DNA fragments of a similar length, resulting in double bands.
However, both effects are insignificant for the evaluation and fundamental comprehension of the methodology.

VI. X. Safety and disposal

The safe handling of laboratory equipment and chemicals requires a certain level of fundamental knowledge and safety measures. As a general rule, you should wear a laboratory coat and safety goggles during the experiment. Gloves should also be provided and worn as required. When preparing the agarose gel, wear insulated gloves in order to avoid burning or scaling your hands.

The handling of the equipment and the risks that are involved should be known. Particular attention must be paid to the electricity hazards. Ensure that all of the connectors, mains power cables and work surfaces (and your hands) are dry prior to operating the electrical equipment.

Further health and safety measures: Tie your hair back, do not wear any jewellery and wear clothes with tight-fitting sleeves in order to avoid any unwanted contact with the equipment, chemicals, etc.

Waste must be disposed of in accordance with the instructions and with the local rules and regulations.

Potential hazards of the components of the kit

DNA samples

The DNA samples include 10% glycerine as well as the dye bromophenol blue with a concentration of 0.25%.
In accordance with the directive (EC) 1272/2008, the substance or mixture has not been rated as hazardous.
In accordance with the directive 67/548/ECC or 1999/45/EC, the substance or mixture has not been rated as hazardous.

Electrophoresis buffer, 50x concentration

The following information refers to the concentrated electrophoresis buffer. This means that it does not necessarily apply to the diluted buffer (working solution).
Rating in accordance with the directive (EC) 1272/2008:
Hazard information
H315: Causes skin irritation
H319: Causes serious eye irritation
H335: May cause respiratory irritation

Safety information
P280: Wear protective clothing and eye protection.
P261: Avoid breathing dust/fume/gas/mist/vapours/spray.
P302+P352: If on skin: Wash with plenty of water and soap.
P305+P351+P338: If in eyes: Rinse cautiously with water for several minutes. Remove contact lenses if present and easy to do. Continue rinsing.

Rating in accordance with the directive 67/548/ECC or with directive 1999/45/EC:
Hazard information
R36+R37+R38: Irritating to eyes, respiratory system and skin.

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Safety information
S26: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.
S28: After contact with skin, wash immediately with plenty of water.
S37+S9: Wear suitable gloves and eye/face protection. Keep container in a well-ventilated place.

Agarose
In accordance with the directive (EC) 1272/2008, the substance has been rated as non-hazardous.
In accordance with the directive 67/548/ECC or 1999/45/EC, the substance or mixture has not been rated as hazardous.
Recommendation: Wear gloves and protective goggles. Avoid contact with the skin and eyes. Avoid the formation of dust. Do not breathe the agarose.

DNA staining solution (200x concentration)
In accordance with the directive (EC) 1272/2008, the aqueous solution has not been rated as hazardous.
In accordance with the directive 67/548/ECC or 1999/45/EC, the substance or mixture has not been rated as hazardous.
Recommendation: Wear gloves and protective goggles. Avoid contact with the skin and eyes.

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