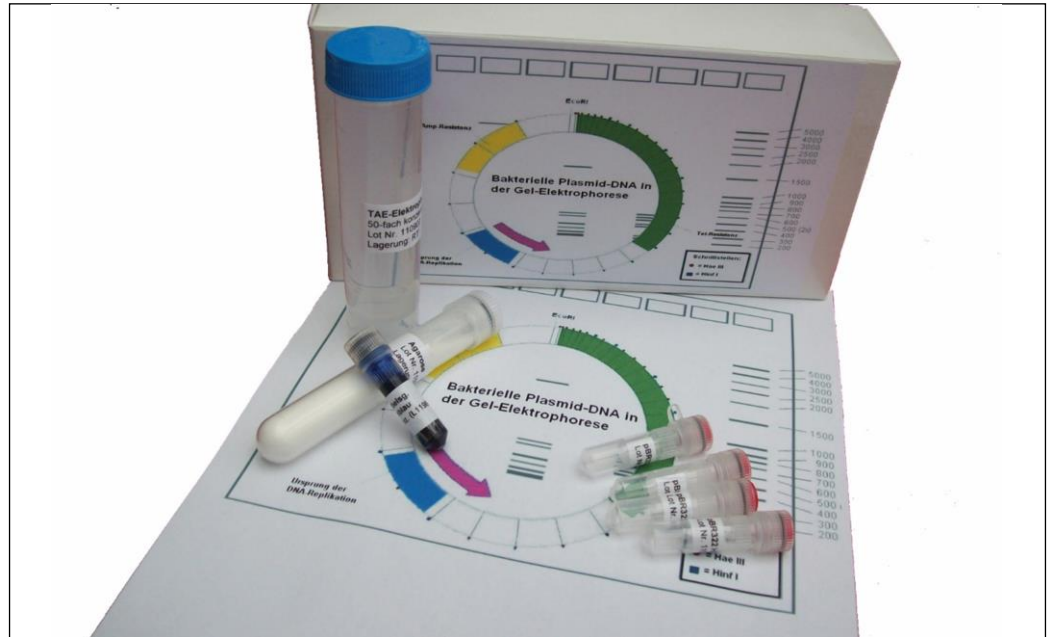


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## Operating manual

[Kit: Bacterial plasmid DNA in gel electrophoresis of] [KLA-530-100]

### ***I. Area of application/introductory remark***

These instructions describe the preparation of plasmid DNA in agarose gel electrophoresis. During the experiment, uncut (circular) plasmid DNA can be compared to plasmid DNA that has already been cut by way of enzymes (linearised DNA).

Naturally, the enclosed uncut DNA can also be used for own restrictions. The required restriction enzymes are not included in the kit. They are available from specialist shops.

The basis of the DNA kit is the plasmid pBR322 which is one of the first plasmids dating back to the 1970s. In the name, p stands for plasmid while B and R stand for Bolivar and Rodriguez, the names of the researchers who constructed it. 322 is a consecutive number of similar, though not identical, plasmids. Other well-known plasmids of this series are pBR325 or pBR328, for example.

Details concerning the plasmids can be found in specialist literature or Internet databases.

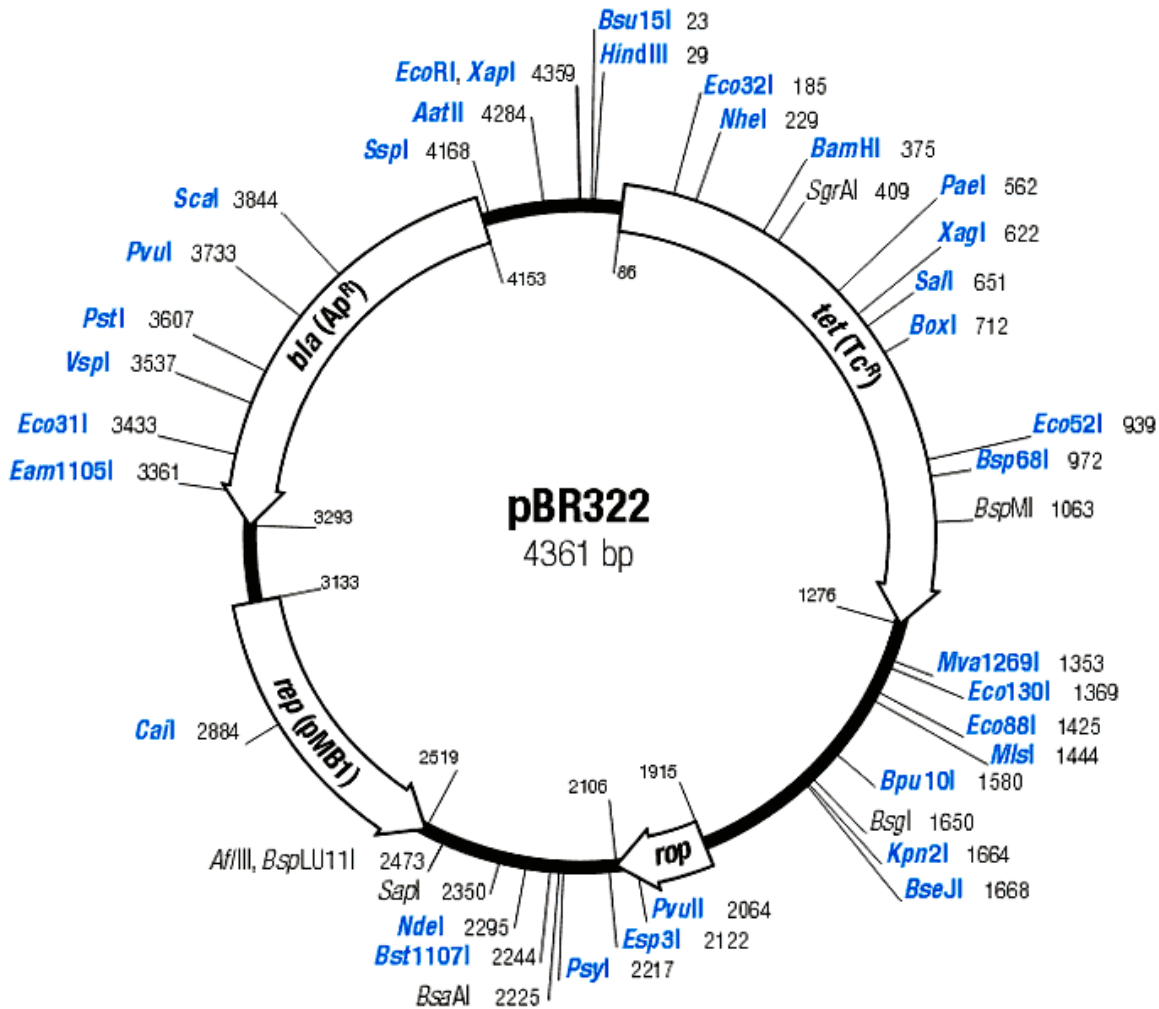


Fig. 1: Schematic representation of the pBR322 plasmid. The origin of replication (rep) and two resistance genes (tet and bla) are shown. In addition, the restriction sites for all rare cutters are also indicated, including EcoRI, however, not of the frequent cutters HinfI and HaeIII.

## II. Content

pBR322 DNA, uncut	10 µg lyophilised
(ccc and oc form visible in the gel)	
pBR322 DNA, EcoRI, cut	10 µg lyophilised
pBR322 DNA, HinfI, cut	10 µg lyophilised
pBR322 DNA, HaeIII, cut	10 µg lyophilised
Gel loading buffer for dissolving the freeze-dried plasmid DNA, contains bromophenol blue as a colour marker	0.5 ml (ready for use)
Agarose	6 g
TAE buffer (50x concentration)	50 ml
DNA staining solution (200x concentration)	1.5 ml

### Note:

The gel loading buffer is used to "load" the DNA molecules so that they can sink into the gel pockets. The dye in the gel loading buffer facilitates the transfer of the buffer into the gel pockets by way of a pipette and it is also used to monitor the progress of the electrophoresis.

Freeze-dried DNA is transparent and, therefore, hardly visible in the vessels (transparent film).

## III. Storage

Due to the fact that the DNA samples have been freeze-dried, they are extremely stable and can be stored at room temperature for a few days. The same applies to the other components of the kit. However, we recommend storing them in the refrigerator. Only the agarose and TAE buffer should be stored at room temperature.

#### **IV. Dissolving the DNA in the gel loading buffer**

Dissolve the DNA in the gel loading buffer in a sterile manner, if possible. The final concentration of the dissolved DNA depends on the sensitivity of the DNA staining.

For staining with methylene blue, as is intended for this kit, the DNA must be dissolved as follows:

pBR322 DNA, uncut	+ addition of 110 µl of the gel loading buffer
pBR322 DNA, EcoRI, cut	+ addition of 130 µl of the gel loading buffer
pBR322 DNA, HinfI, cut	+ addition of 110 µl of the gel loading buffer
pBR322 DNA, HaeIII, cut	+ addition of 110 µl of the gel loading buffer

The dissolving process takes approximately 15 minutes. Shake the vessels vigorously in between on a laboratory shaker or by hand. At the end of the dissolving process, spin them briefly or bounce the vessels briefly on the desktop so that the entire liquid collects at the bottom of the vessel.

**Tip:** The dissolution of the DNA can also take place overnight in a refrigerator so that the dissolved DNA will be available the next day.

#### **V. Casting the agarose gel**

1.4% agarose gel is most suitable for the preparation of the PCR products. It can be prepared with the supplied TAE electrophoresis buffer (50x concentration). The buffer must be diluted with distilled water at a ratio of 1:50 beforehand.

1. Weigh the required quantity of agarose for a 1.4% gel and fill it into an Erlenmeyer flask.
2. Measure the corresponding volume of the 1x concentrated electrophoresis buffer in a graduated cylinder and add it to the agarose.
3. Heat the agarose in a microwave oven or in boiling water until it has completely liquefied. Remove the Erlenmeyer flask repeatedly in between and shake it in order to ensure the homogeneous fusion of the agarose.  
**Caution:** Wear kitchen gloves when holding the hot Erlenmeyer flask. In addition, superheating (boiling retardation) may occur so that the agarose may splash out of the flask (protective goggles!).
4. Let the liquid agarose cool down to approximately 80°C. Then, cast the gel.
5. Place a comb in the cast and let the agarose gel solidify (approximately 20 minutes).
6. Overlay the gel with the electrophoresis buffer and carefully remove the comb.
7. The teeth of the comb have created gel pockets in the solid agarose. Later, the samples will be filled into these pockets by way of a pipette.

**Tip:** If you want to wait until the next day for performing the actual electrophoresis, leave the comb in the gel, overlay the gel with the electrophoresis buffer and cover everything with cling film so that the gel will not dry up.

**Note:**

The electrophoresis buffer can be used several times. Store it at room temperature.

#### **VI. Applying the samples for gel electrophoresis**

Fill 10 µl of the various plasmid samples as well as of the DNA length marker into the pockets of the agarose gel. Insert the tip of the micropipette into the pocket, but ensure to not pierce the bottom of the pocket. Then, let the sample "flow" carefully into the pocket. The samples that are dissolved in the gel loading buffer are heavier than the electrophoresis buffer so that they sink into the pockets easily.

**Attention:**

Please note that the above-mentioned pipetting recommendations refer to standard mini- or midi-size gel formats with a pocket width of approximately 3.5 mm and a migration length of approximately 50 mm.

The volumes that are stated in section IV apply to staining with methylene blue. If you want to perform more sensitive DNA staining, the volumes can be reduced accordingly.

We recommend using a test gel prior to the actual experiment in order to see whether your volumes are suitable for your gel format. If not, adapt the volumes accordingly.

The samples that have been prepared for the electrophoresis can be stored in the refrigerator for several days if you want to perform the actual electrophoresis at a later time.

#### **VII. Agarose gel electrophoresis**

In general, a migration distance of 5 to 8 cm is sufficient for separating the corresponding DNA fragments. The dye bromophenol blue in the gel loading buffer "indicates" the progress of the separation. The electrophoresis will be stopped when the colour marker bromophenol blue has reached 2/3 to 3/4 of the gel length.

Connect the electrophoresis chamber to a suitable voltage supply unit. The electrophoretic separation of the DNA samples takes place at a voltage of approximately 3 to 5 Volts/cm (distance between electrodes) when using a TAE electrophoresis buffer.

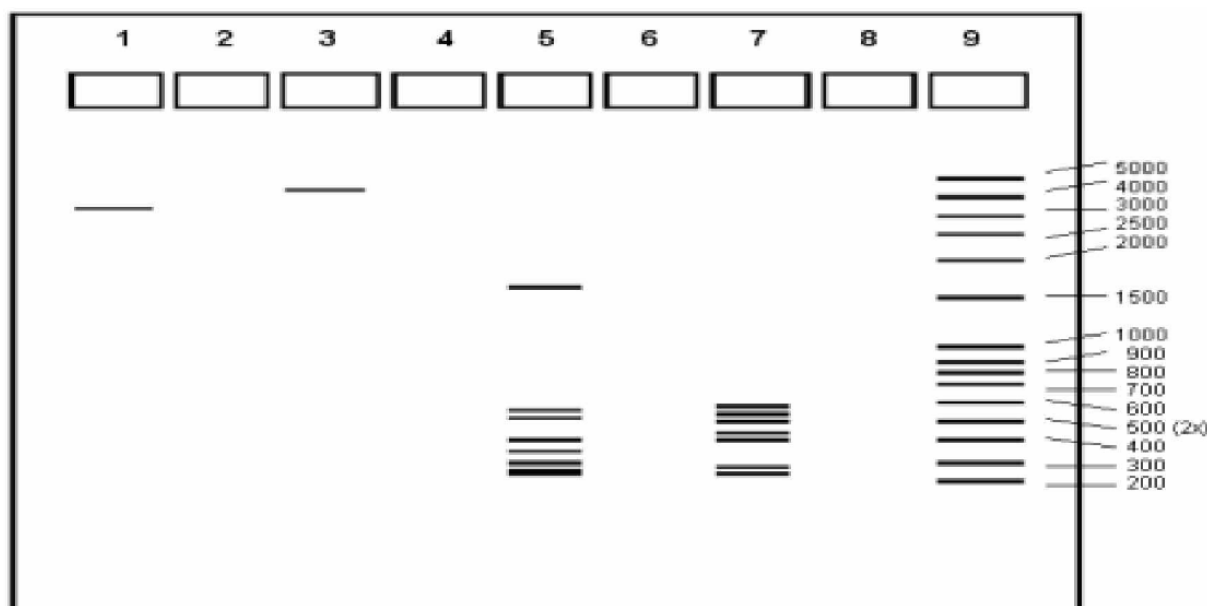
Please refer to the information and notes that are provided in the operating instructions of your electrophoresis apparatus. Your apparatus may not be suitable for the above-mentioned voltages. In this case, a lower voltage must be used.

### VIII. Staining the agarose gel

1. Prepare the DNA staining solution by diluting 1 ml of the staining concentrate with 200 ml of distilled water. The staining solution can be used several times. Stored in a closed container at 4°C in a refrigerator.
2. After the electrophoresis, transfer the agarose gel carefully with a kitchen spatula into a gel staining dish and pour the staining solution over the gel.
3. Stain the gel for approximately 10 to 15 minutes. Shake the dish from time to time during this process.
4. Then, fill the staining solution into a labelled bottle for storage and store it in the refrigerator. Next, destain the gel with tap water until the background is sufficiently light so that the stained DNA bands contrast well with the background.
5. The blue DNA bands can be documented by taking a photo of the gel with transmitted light (e.g. by way of a light box for slide presentations).

#### Tip:

We recommend storing the gel overnight in a refrigerator, since the staining of the DNA bands usually intensifies. Wrap the gel into Saran wrap or store it in a small plastic bag. If necessary, the gel can be further destained the day after it has been stained if the background is still too blue.



**Fig. 2: Electrophoretic separation of pBR322 DNA (stained with methylene blue):**

Lane 1: pBR322, undigested (ccc form); lane 3: pBR322 EcoRI; lane 5: pBR322 Hinf I; lane 7: pBR322 Hae III; lane 9: optional DNA size marker, applied in addition (not included in the kit as standard). All values in base pairs (bp).

**Note:** The undigested plasmid pBR322 (ccc form: covalently closed circle) migrates more quickly through the gel than the linearised plasmid which was digested by EcoR1 at one position. The "balled" conformation of this plasmid encounters less resistance in this separation matrix which is why it displays slightly higher mobility in the gel.

**Fragment lengths that occur after the corresponding restriction digests (values in base pairs (bp)):**

pBR322-DNA EcoRI	pBR322-DNA HinfI	pBR322-DNA HaeIII
4.361	1.632	587
	517	540
	504	502
	396	458
	344	434
	298	267
	221	234
	220	213
	154	192
	75 (1)	184
		124
		123
		104
		89
		80,64,57
		51,21,18,
		11, 8 (2)

(1): Only visible with optimum loading and sensitive staining.

(2): Individual, low-molecular fragments no longer separate in most cases.

Bromophenol blue cannot sufficiently stain fragments below 200 bp, since the dye is not as sensitive as the fluorescent dye ethidium bromide. However, this can be tolerated and has only a minor influence on the evaluation.

### ***IX: Practical tips for the lessons/lab courses***

DNA experiments with this kit system can be easily integrated into the lessons/lab courses since the experiment can be interrupted in between. You can interrupt it at various times, store the samples in a refrigerator and continue when it suits you. Interruptions are possible, for example, after you have completed the steps that are described in sections IV and V. However, the staining step should be performed directly after the actual gel electrophoresis (section VII) since, otherwise, the DNA bands will diffuse in the gel, causing them to become blurry.

### ***X. Safety and waste 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**

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.

#### **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.