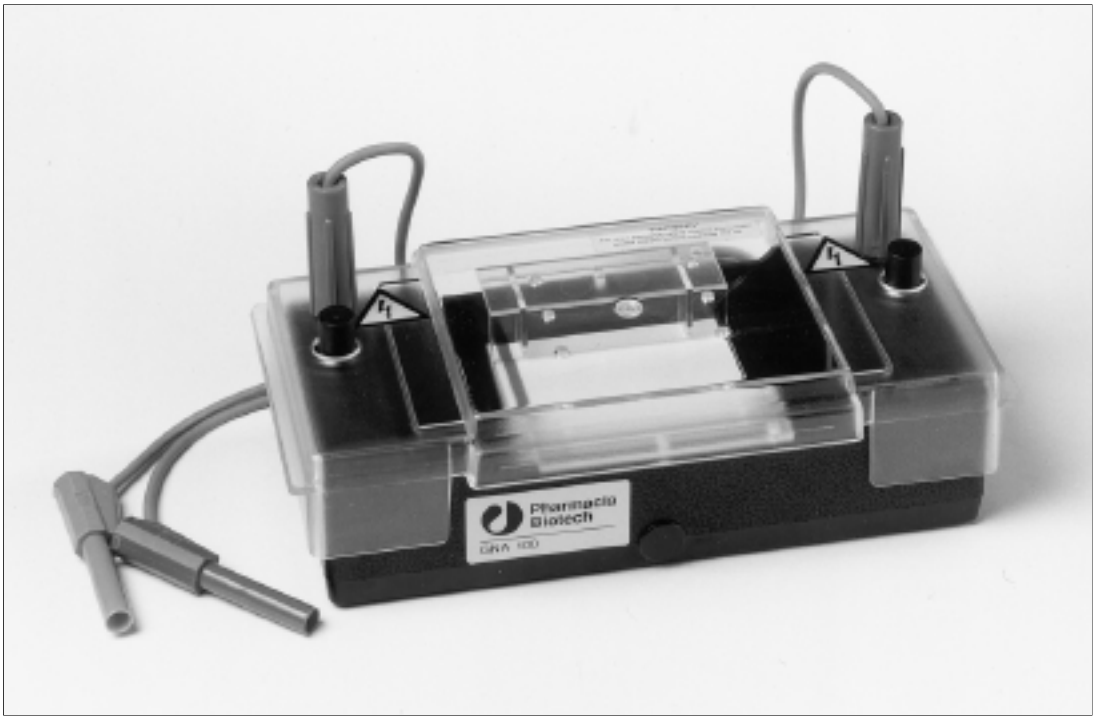
AMERSHAM BIOSCIENCES

Gel Electrophoresis Apparatus GNA-100



User Manual

56-2434-01
Edition AH

Amersham Biosciences

Important user information

Reading this entire manual is recommended for full understanding of the use of this product.

Meaning: Consult the instruction manual to avoid personal injury or damage to the product or other equipment.

WARNING!

The Warning sign is used to call attention to the necessity to follow an instruction in detail to avoid personal injury. Be sure not to proceed until the instructions are clearly understood and all stated conditions are met.

CAUTION!

The Caution sign is used to call attention to instructions or conditions that shall be followed to avoid damage to the product or other equipment. Be sure not to proceed until the instructions are clearly understood and all stated conditions are met.

Note

The Note sign is used to indicate information important for trouble free or optimal use of the product.

Should you have any comments on this manual, we will be pleased to receive them at:

Amersham Biosciences
S-751 82 Uppsala
Sweden

Amersham Biosciences reserves the right to make changes in the specifications without prior notice.

Warranty and Liability

Amersham Biosciences guarantees that the product delivered has been thoroughly tested to ensure that it meets its published specifications. The warranty included in the conditions of delivery is valid only if the product has been installed and used according to the instructions supplied by Amersham Biosciences.

Amersham Biosciences shall in no event be liable for incidental or consequential damages, including without limitation, lost profits, loss of income, loss of business opportunities, loss of use and other related exposures, however caused, arising from the faulty and incorrect use of the product.

Trade marks

Gel Electrophoresis Apparatus GNA-100™ is the exclusive trade marks of Amersham Biosciences. In view of the risk of trade mark degeneration, it is respectfully suggested that authors wishing to use these designations refer to their trade mark status at least once in each article.

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Contents

1. Introduction

Electrophoresis through horizontal agarose gels has become the standard method used to separate, identify and purify nucleic acids and fragments. The technique is simple to perform and capable of resolution and sensitivity unequalled by alternative procedures.

Gel Electrophoresis Apparatus GNA-100 is designed for electrophoresis of nucleic acids in horizontal agarose mini-gels. Electrophoresis is most commonly carried out in the "submarine" mode, in which the gel is just submerged beneath the surface of the electrophoresis buffer. GNA-100 is also suitable for running gels with paper wicks. The mini-gel format means that electrophoresis times are short and that very small amounts of material may be detected. Gels may be prepared and run on either of two UV transparent gel trays or on standard glass plates.

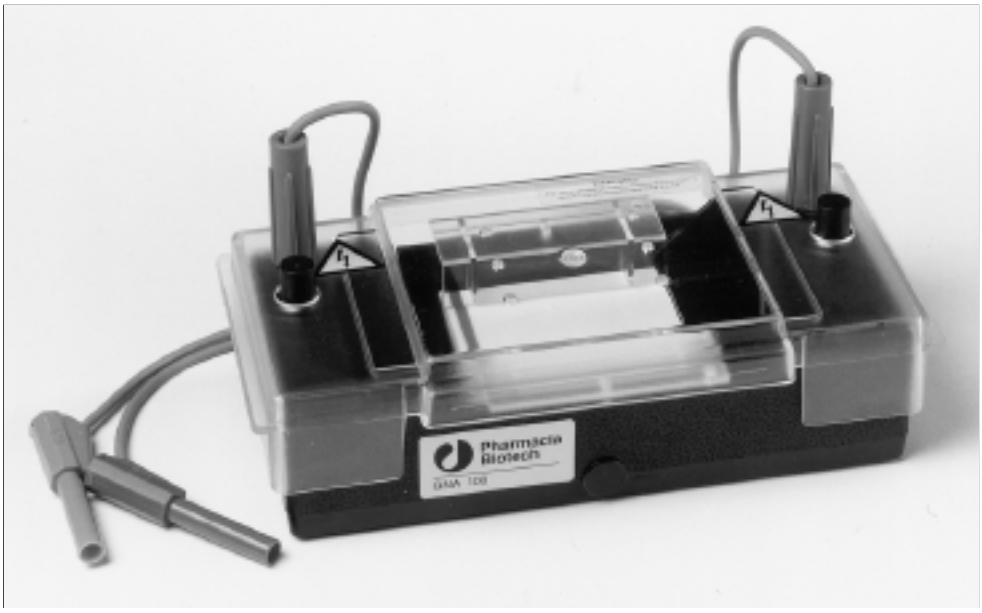

Fig. 1. The Gel Electrophoresis Apparatus GNA-100

2. Important safety information

IMPORTANT SAFETY INFORMATION

To avoid any risk of injury, the instrument should only be operated by properly trained personnel and always in accordance with the instruction provided. Read this entire manual before using the instrument.

WARNING! This instrument is designed for indoor use only.

WARNING! Do not operate the instrument in extreme humidity (above 95% RH). Avoid condensation by equilibrating to ambient temperature when taking the unit from a colder to a warmer environment.

WARNING! Always check the wires for damage before using the unit.

WARNING! Always check that the electrodes are properly connected before closing the lid.

WARNING! Always connect the lid according to the mounting instruction.

WARNING! Always connect the cables to the power supply BEFORE turning the power supply ON.

WARNING! Always TURN OFF the power supply before removing the lid.

WARNING! Do NOT use concentrated acids, bases or halogenated and aromatic hydrocarbons.

WARNING! Never exceed maximum allowed voltage, current or power.

GNA-100 is specifically designed with a safety interlocking system so that no part of the active electrophoresis chamber is exposed during operation.

Do not attempt to change or modify this safety design.

3. Description of the system

3.1 The gel box

The gel box is made of black Bayblend* plastic and is of a moulded construction. This eliminates the possibility of buffer leakage from box joints.

The thumb supports (17,18) at either end of the box are to assist in removal of the lid (Fig. 4). The gel box is supported on four feet.

3.2 The electrodes

GNA-100 features a platinized titanium cathode (2) and anode (3). Both electrodes are rods of 1.5 mm diameter which are rigid enough not to be distorted during normal usage of the apparatus. The portion of each electrode running vertically downwards from the electrical connector is covered with insulating material in order to eliminate distortions of the electrical field. Should removal of an electrode be necessary this may be achieved by unscrewing the electrical plug (19, 20) and then unclipping the horizontal part of the electrode from the bottom of the box.

3.3 The lid

The lid is moulded from UV transparent acrylic material. HT cables are attached to the lid by female connectors. Holes in the lid close to the connectors are for positioning of the thumb supports (17,18). Six further small holes are to allow access for probes for the measurement of temperature and electrical field strength.

3.4 The gel tables

The two gel tables (8,11) are made of Bayblend. There are fixing lugs on the underside of each table. Gel tables are fitted into the gel box by clipping the lugs onto the partition between the two buffer chambers.

The upper surface of each gel table is coloured mainly white. There are red stripes (23) across the plate which lie directly underneath the sample wells when a gel is in the apparatus. The red stripes allow the wells to be easily distinguished when loading samples and the white background allows easy visualization of tracker dyes during electrophoresis.

3.5 The gel tray

The gel trays are moulded from acrylic material which is transparent to UV light. A series of holes in the upper edge of the tray sides are used to position the gel comb(s). Locating pins on the under surface of the tray sides allow a tray to be correctly positioned in the gel box.

3.6 The gel combs

The gel combs provided with GNA-100 are moulded from black Noryl** plastic. The space between the bottom of the wells and the gel plate depends upon which way the gel comb is positioned in the tray. This distance may be changed from approximately 1 mm to 2 mm (or vice versa) by turning the comb through 180° horizontally.

In addition to the gel combs supplied, a number of other combs are available (see section 8, Ordering information and technical data).

* Bayblend is a registered trade mark of Bayer A.G., Germany.

** Noryl is a registered trade mark of General Electric Company, USA

4. Installation

Description

GNA-100 contains the components listed in Table 1. Components are numbered in accordance with the diagram of the apparatus shown in Fig. 4 on page 16.

Table 1. GNA-100 components

Number	Designation	No. supplied
1	Gel box with	1
2,3	electrodes	2
4	feet	4
5	Lid with	1
	power cables	2
8	Gel table I	1
9	Gel tray I	1
10	Gel comb (11 teeth, 3x1 mm) for gel tray I	1
11	Gel table II	1
12	Gel tray II	1
13	Gel comb (7 teeth, 3x1 mm) for gel tray II	1

5. Operation

5.1 Using the GNA-100

The most common use of GNA-100 is for the separation of DNA fragments. This application will be described in the following sections of the manual. Manipulations of gels after electrophoresis and staining, as well as electrophoresis of RNA, are beyond the scope of this manual. Description of such techniques can be found in many publications on molecular biology methodology.

Before making a gel, consideration should be given to agarose type and concentration, to the type of electrophoresis buffers to be used and to the gel volume.

5.1.1Agarose type

Many different grades of agarose are available but not all are suitable for electrophoresis of nucleic acids. For most nucleic acid electrophoresis the requirement is for a low electroendosmosis agarose which produces gels of high strength and optical transparency. However, many agaroses of this type contain contaminates which inhibit enzymes such as ligases and restriction endonucleases. Therefore, DNA fragments eluted from such gels have to be extensively purified before they can be used as substrates for these enzymes.

Amersham Biosciences Agarose NA is a low electroendosmosis agarose which produce gels of high strength and optical clarity. Furthermore, this agarose is specially tested and selected for low inhibition of the action of ligase and restriction enzymes. (See section 8, Ordering information and technical data, Companion products.)

5.1.2Agarose concentration

The mobility of nucleic acids in agarose gels depends upon the concentration of agarose present. By using gels of different concentrations it is possible to separate a wide size range of nucleic acid fragments. Table 2 shows the efficient separation range of agarose gels of various percentages.

Table 2. Efficient separation range of agarose gels of various concentrations.

Agarose concentration in gel (%)	Efficient separation range for linear DNA molecules (kb)
0.3	60-5
0.6	20-1
0.7	10-0.8
0.9	7-0.5
1.2	6-0.4
1.5	4-0.2
2.0	3-0.1

Gels of low agarose concentration (0.5% or less) or of chemically modified agarose (low melting agaroses) are particularly subject to the effect of electrical heating. Distortion of DNA bands may occur unless gels are run at lower than normal voltages (2 V/cm or less).

5.1.3Type of buffers

Buffers containing Tris-acetate, -borate, or -phosphate are all commonly used in conjunction with horizontal agarose gels. There is considerable variation between laboratories in the exact formulation of buffers used. Table 3 shows some typical examples.

Table 3. Composition of commonly used buffers.

Buffer	Working solution stock solution (per litre)	Composition of concentrated	
Tris-acetate (TAE)	40 mM Tris-acetate 2 mM EDTA	50x:	242 g Tris base 57.1 ml glacial acetic acid 200 ml 0.5 M EDTA (pH 8.0)
Tris-phosphate (TPE)	89 mM Tris-phosphate 2 mM EDTA	10x:	108 g Tris base 15.5 ml of 86% phosphoric acid (1.679 mg/ml) 40 ml 0.5 M EDTA (pH 8.0)
Tris-borate (TBE)	89 mM Tris-borate 89 mM Boric acid 2 mM EDTA	5x:	54 g Tris base 27.5 9 boric acid 20 ml 0.5 M EDTA (pH 8.0)

Tris-acetate is the most frequently used buffer for general electrophoresis of DNA in agarose gels. However, it has a rather low buffering capacity and recirculation of buffer between the electrode compartments is recommended.

Gel thickness: For general analytical separations a gel of 0.4 cm thickness might typically be used. This means a gel volume of approximately 30 ml when using Gel Tray I and 16 ml when using Gel Tray II.

Gels for GNA-100 may be prepared either directly in one of the gel trays or on 50x75 mm glass plates.

5.2 Making a gel in a gel tray

1. Make sure that the gel tray is clean and dry before use. Seal the ends of the gel tray with Amersham Biosciences tape (see Companion Products) or other similar tape. Press the tape firmly to the edges of the tray to ensure a tight seal.
2. Place the gel comb(s) in position in the gel tray and place the tray on a Amersham Biosciences levelling table (see Companion Products) or other horizontal surface.
3. Place the required amount of agarose powder into an Ehrlenmeyer conical flask and add the appropriate volume of electrophoresis buffer (diluted to running concentration). Cover the flask.
4. Dissolve the agarose by heating either on a laboratory heating block, in a boiling-water bath, in a microwave oven, or in an autoclave. Make sure that all the agarose particles are completely dissolved before proceeding further.

5. Cool the agarose solution to between 50°C and 60°C. If you wish to carry out electrophoresis with ethidium bromide in the gel, add the stain to a final concentration of 0.5 mg/ml at this stage.

   Note: The ethidium bromide must also be included in the running buffer at a concentration of 0.5 µg/ml. See section 6. Evaluation and presentation of data.

6. Pour the agarose solution into the gel tray.
7. After the gel is completely set (approximately 30 min at room temperature), carefully remove the gel comb. This may be facilitated by gently rocking the comb backwards and forwards by a few millimeters while pulling upwards. When handling soft gels (0.6% agarose or less) it is advisable to flood the surface of the gel with buffer before removing the comb. This reduces the possibility of damaging the sample wells.
8. Remove the tape from the ends of the tray and put the tray into position in the gel box.

9a. For electrophoresis in the “submarine” mode, pour enough electrophoresis buffer into the apparatus to cover the gel to a depth of at least 1 mm.

9b. For electrophoresis with wicks, cut the wicks to the exact width of the gel, wet them with electrophoresis buffer and place them so that they overlap the gel by about 1 cm. Pour 100 ml of buffer into each buffer chamber.

5.3 Making a gel on a glass plate

Gels may be prepared on standard glass plates (see Spare parts and accessories) without the use of tape. Surface tension effects prevent molten agarose from flowing off the plate, provided the agarose layer is not more than 3.5 mm thick. For this application Gel Tray I is used to fix Gel Comb II in position over the glass plate.

1. Wipe the glass plate with 70% ethanol or methanol to remove traces of dirt and grease.

2. Place the Gel Tray I on a Amersham Biosciences levelling table (see Companion Products) or other horizontal surface. Position the cleaned glass plate and gel comb on the gel tray as shown in Fig. 2.

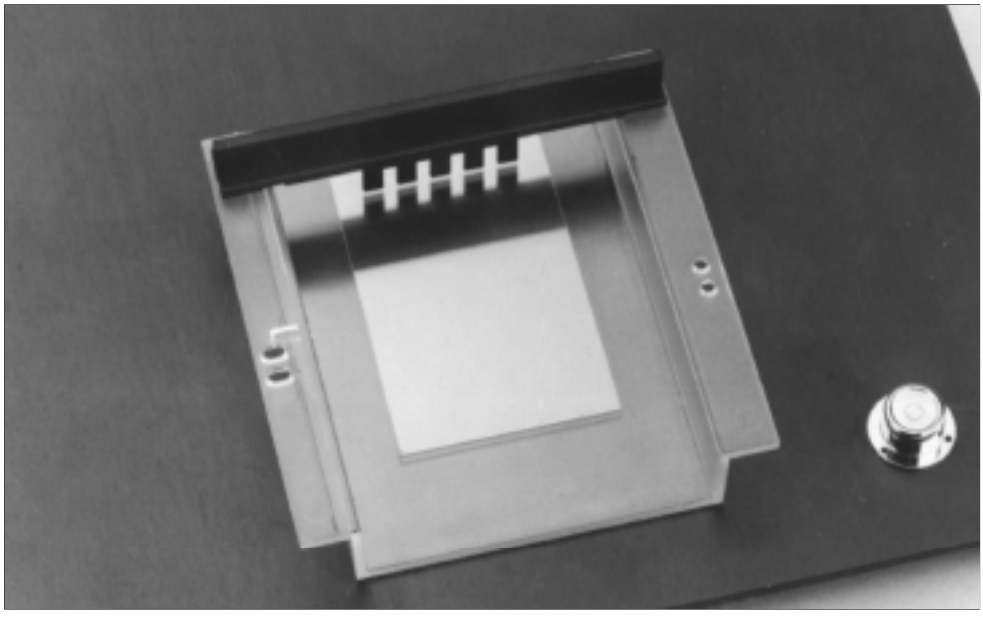

Fig. 2. Positioning a glass plate in the gel tray.

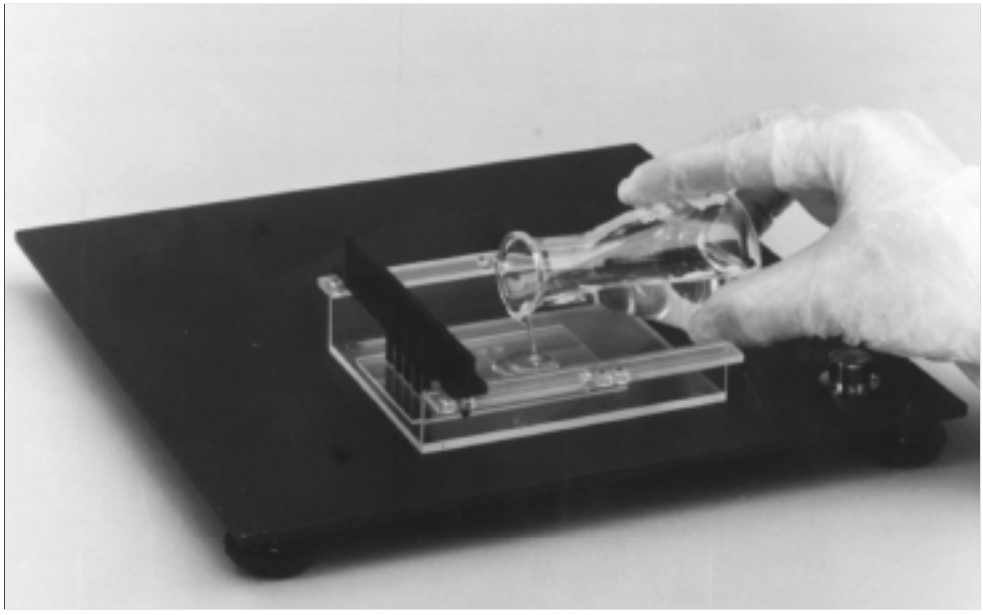

Fig. 3. Pouring a gel.

Note: The gel comb should be positioned such that there is a space between the bottom of each tooth and the glass plate.

3. Prepare and cool 10 ml of agarose solution using the method described in Section 5.2, 3-5. Carefully pour the agarose onto the centre of the glass plate (Fig. 3). The thickness of the agarose will be 2.5 mm.
4 After the gel is completely set (approximately 10 min at room temperature) carefully remove the gel comb.
5. Carefully remove the gel, on the glass plate, from Gel Tray I and place it into position, on Gel Tray II, in the GNA-100 apparatus.
6. Pour enough electrophoresis buffer into the apparatus to cover the gel to a depth of at least 1 mm.

5.4 Sample preparation and loading

Sample volume

The maximum sample volume which can be applied in a well depends upon the thickness of the gel used. When using a 4 mm thick gel with wells 3 mm deep and 3 mm wide, the volume of each well is 9 µl. Remember to make allowance for the addition of loading buffer to samples before application (commonly, the volume of loading buffer added is one fifth of the original volume of the sample).

Amount of sample

The maximum amount of DNA that can be applied to a well depends upon the number and size of fragments in the sample. For a 3 mm wide well the maximum amount of DNA which may be separated as a single band without smearing or trailing is about 50 ng. This means that 0.05-0.5 µg of a simple population of DNA molecules may be loaded in a 3 mm wide well. However, when samples consist of many fragments of different sizes (e.g. restriction digests of whole mammalian genomes) it is possible to load up to 5 µg per slot without serious loss of resolution.

The minimum amount of DNA which can be detected in a single band by photography of ethidium bromide stained gels is approximately 2 ng in a 3 mm wide well.

Sample preparation

The loading buffer concentrate (6x) consists of 0.25% bromophenol blue, 0.25% xylene cyanol and 15% Amersham Biosciences Ficoll 400 dissolved in water. The dyes allow the progress of electrophoresis to be monitored and Ficoll 400 makes the samples dense for underlayering into sample wells. Prepare samples for loading by adding one fifth volume of concentrated loading buffer.

Sample loading

Load samples into the sample wells slowly, using a disposable micropipette or an automatic micropipettor. Be careful to avoid damaging the wells with the pipetting device. It is not necessary to position the tip of your pipette close to the bottom of a sample well as Ficoll 400 makes the sample dense enough to fall to the bottom of the well.

5.5 Connecting the system

Carefully place the lid on the gel box, passing the thumb supports through the holes in the lid. The female connectors on the lid should be pushed down as far as possible onto the male connectors on the box. Connect the free ends of the power cables to a power supply such as Amersham Biosciences EPS Power Supply.

The red connector should be attached to the positive terminal of the power supply and the black connector should be attached to the negative terminal.

CAUTION! Do not connect the apparatus to a power supply which is capable of delivering more than 500 V.

5.6 Electrophoresis

At the pH used for electrophoresis, nucleic acids have a net negative charge. They will, therefore, migrate towards the positive electrode (anode) during electrophoresis. You should make sure that the gel apparatus is connected so that the negative electrode (cathode) is nearest to the sample wells at the start of electrophoresis.

Sample:	Mixed lambda DNA-Hind III/øX-174 RF DNA Hinc II fragments.
Gel:	Agarose NA, 4 mm thick.
Buffer:	50 mM Tris acetate, 5 mM EDTA (pH 8.0).
Electrophoresis conditions:	65 V, 37 mA (initial).
Run time:	2 hours.

Table 4. Typical separation of DNA fragments on GNA-100.

Fragment size in base pairs	Migration distance in mm
23130	14
6557	21
2027	38
1057	49
564	61

WARNING! Always TURN OFF the power supply before removing the lid.

After electrophoresis, switch off the power supply and disconnect GNA-100. Remove the lid by gripping it under the rim with finger tips and pressing down on the thumb supports.

6. Evaluation and presentation of data

6.1 Detection of nucleic acids in agarose gels

The most convenient method of visualizing DNA in agarose gels is by staining with the intercalating fluorescent dye ethidium bromide. DNA-ethidium bromide complexes absorb ultraviolet light at 260, 300 or 360 nm and emit at 590 nm in the red-orange region of the visible spectrum. Sensitivity of detection decreases with illumination at higher wavelengths. However, illumination below 300 nm may cause significant damage to DNA which may be disadvantageous if fragments are to be further manipulated. Furthermore, UV light can also cause damage to skin and eyes and should not be used without protection. Ethidium bromide can be used to detect both single- and double-stranded nucleic acids (both RNA and DNA). However, the affinity of the dye for single-stranded nucleic acid is relatively low and fluorescence in ultraviolet light is therefore poor.

Ethidium bromide is a powerful mutagen and should be handled with care. Always wear non-permeable gloves when handling gels or solutions containing the dye. Dispose of ethidium bromide according to local rules governing hazardous chemical waste.

A. Ethidium bromide may be incorporated directly in the gel during its preparation (see Making a gel). In this case the dye must also be present (at 0.5 µg/ml) in the running buffer during electrophoresis. The presence of ethidium bromide during electrophoresis reduces the mobility of linear duplex DNA by about 15%. On the other hand, if ethidium bromide is present, the separation of molecules may be directly monitored under UV illumination during the course of electrophoresis.

B. Agarose gels may also be run in the absence of ethidium bromide and stained after electrophoresis. After electrophoresis remove the gel from the apparatus on the gel tray. Immerse the gel, on the gel tray, in electrophoresis buffer containing ethidium bromide (1 µg/ml) for 15-30 minutes. Destaining is not usually essential although background fluorescence may be reduced by briefly washing the gel in water (10 minutes or less).

After staining, gels are viewed by illumination with ultraviolet light from either above or below (see previous section). Photography of gels under UV illumination is commonly carried out on Polaroid Type 667 film (ASA 3000) in a camera fitted with a red-orange filter (Kodak 22A Wratten or similar) . With this kind of system an exposure of a few seconds is sufficient to obtain images of bands containing as little as 10 ng of DNA. Longer exposure under a strong source can allow detection of as little as 1 ng of DNA.

For documentation and analysis of results, a gel documentation system, such as the Amersham Biosciences ImageMaster® VDS System, is recommended.

7. Maintenance

CAUTION! Clean the apparatus by washing with water and a mild soap or detergent. Rinse well with distilled water and dry with a soft tissue or cloth. Avoid the use of abrasive cleaners and rough cloths or brushes. Do not dry or sterilize the apparatus at high temperatures.

Grease and adhesive from sealing tape may be removed by gently wiping with hexane or paraffin. Do not use acetone, halogenated hydrocarbons or undiluted laboratory alcohols (over 30%) for cleaning.

8. Ordering information and technical data

8.1 Ordering information

Spare parts and accessories

Components are numbered in accordance with the diagram of the apparatus shown in Fig. 4.

Number	Designation	Code No.	No. per pack
2	Cathode GNA-100 (Platinized titanium)	18-9443-01	1
3	Anode GNA-100 (Platinized titanium)	18-2412-01	1
5	Lid GNA-100 (include HT cables and connectors	18-1121-73	1
8	Gel table I	18-2403-01	1
9	Gel tray I	18-2409-01	1
11	Gel table II	18-2404-01	1
12	Gel tray II	18-2410-01	1
19, 20	Plug 4 mm	18-2319-01	1
	Glass plates (50x75 mm)	18-2424-01	10
	Combs for Gel tray I:		
	Analytical comb, 11 wells (3x1 mm)	18-1019-42	1
	Analytical comb, 8 wells (5x1 mm)	18-1019-43	1
	Analytical comb, 8 wells (5x2 mm)	18-1019-44	1
	Analytical comb, 5 wells (10x1 mm)	18-1019-45	1
	Prep. comb, 2 small (3x1 mm) and 1 large (54x1 mm) well	18-1019-46	1
	Prep. comb, 2 small (3x2 mm) and 1 large (54x2 mm) well	18-1019-47	1
	Combs for Gel tray II:		
	Analytical comb, 7 wells (3x1 mm)	18-1019-48	1
	Analytical comb, 5 wells (5x1 mm)	18-1019-49	1
	Prep. comb, 2 small (3x1 mm) and 1 large (30x1 mm) well	18-1019-50	1
	Prep. comb, 2 small (3x2 mm) and 1 large (30x2 mm) well	18-1019-51	1

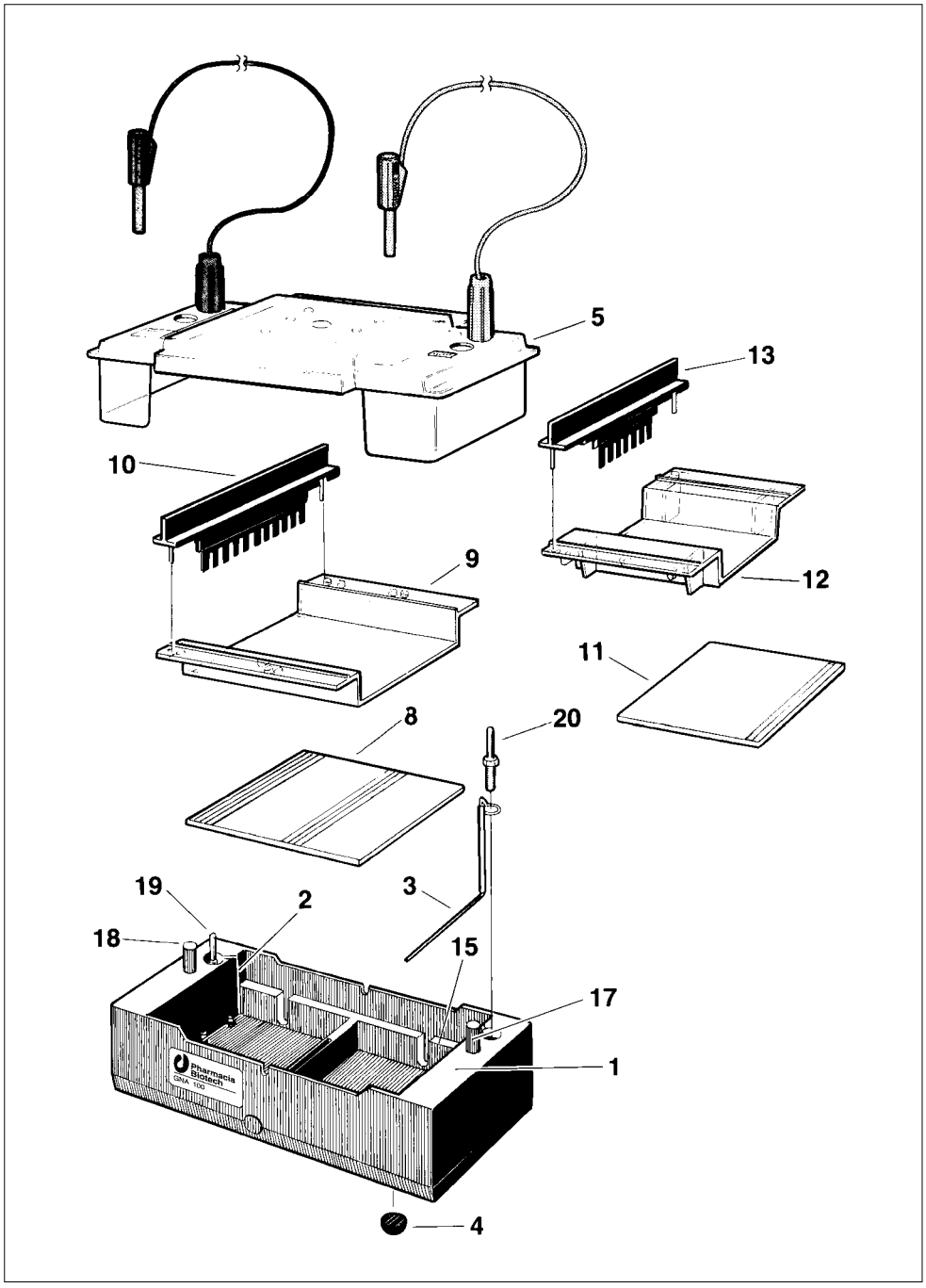

Fig. 4. Exploded diagram of system components.

Companion Products

Designation		Code No.
EPS 200 Power Supply		19-0200-00
MacroVue UV-20 Transilluminator 115 V		80-6245-11
MacroVue UV-20 Transilluminator 230 V		80-6245-30
ImageMaster VDS,	DU 115 VAC, 60 Hz	80-6246-82
	DE 230 VAC, 50 Hz	80-6247-01
	DJ 100 VAC, 50/60 Hz	80-6247-20
ImageMaster VDS Analysis Kit		80-6309-71
AgaroseNA	10 g	17-0554-01
	100 g	17-0554-02
	1 kg	17-0554-03
Agarose Prep 50 g		80-1130-07
Ethidium Bromide, 10 mg/ml solution, 10 ml		17-1328-01
Ficoll 400 100 g		17-0400-01
Tape (66 m)		19-1257-01
Spirit level		80-6087-60

CAUTION! Only spare parts approved or supplied by Amersham Biosciences may be used for maintaining or servicing of GNA-100.

8.2 Technical data

Specifications of GNA-I00

Gel dimensions:	76x100 mm (Gel tray I)	
	52x76 mm (Gel tray II)	
Gel thickness range:	2-6 mm (on gel tray)	
	2-3.5 mm (on glass plate)	
Buffer volume:	with Gel Tray I 300 ml	
	with Gel Tray II 350 ml	
	with Paper Wicks 200 ml	
Electrical range:	Voltage	0-300 V
	Current	0-660 mA
	Maximum power	200 W
Environment	+4-40 °C	
	20-95% relative humidity	
Maximum safety temperature of all materials:	60 °C	
Ultraviolet transparency:	The lid and gel tray are transparent to UV light in the 300 to 360 nm range.	
Chemical resistant:	Resistant to aqueous solvents in the pH 2-11 range.	
	Not resistant to acetone, phenol, halogenated hydrocarbons or undiluted laboratory alcohols over 30%.	
Safety standards:	This products meets the requirements of the Low Voltage Directive (LVD) 73/23/EEC through the harmonised standards EN61010-1	
	Note: The declaration of conformity is valid for the instrument when it is – used in laboratory locations – used in the same state as it was delivered from Amersham Biosciences except for alterations described in the user manual – used as a “stand alone” unit or connected to other CE labelled Amersham Biosciences instrument or other products as recommended.	

Notes:

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Printed in Sweden by TK i Uppsala, Jan 1997