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INSTRUCTION MANUAL OF AGAROSE GEL ELECTROPHORESIS KIT

(KT-AG001/ KT-AG005/ KT-AG010)

Manufactured and Marketed by:

TechResource

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Kit components

Sr.No.	Components	Store at
1.	Buffered agarose (1%)	4°C
2.	5X Tank buffer	4°C
3.	Loading cum dissolving dye with EtBr	4°C
4.	EtBr (10 mg/mL)	4°C
5.	KMnO4 (powder)	RT
6.	D/W	RT

Equipments and consumables required but not provided in the kit:

-Horizontal gel electrophoresis apparatus [maximum gel size 7 (w) X 10 (l) cm]
-DNA/RNA samples
-Glass pipettes (1, 5, 10 mL) each 1 no.
-One 100 mL measuring cylinder
-Two 250 mL beakers
-Hot water bath
-Power supply
-Transilluminator
-Micropipettes-
0.5-10 µL
5-50 µL
10-100 µL
100-1000 µL
-Micropipette Tips

Horizontal electrophoresis apparatus

Electrophoresis apparatus manufactured those marked as * are suitable for this kit.

1) *Sleeksub (TechnoSource)	Gel size - 7 (w) X 10 (l) Cat. no. 08-00102/105
2) Gelmariner (TechnoSource)	Gel size - 10 (w) X 15 (l) Cat. No. 10-00152/155/156
3) Greyhound (TechnoSource)	Gel size - 15 (w) X 20 (l) Cat. no. 09-00202/5/6/3
4) Submariner (TechnoSource)	Gel size - 23 (w) X 20 (l) Cat. no. 17-00232/5/6/3
5) Greywhale (TechnoSource)	Gel size - 30 (w) X 30 (l) Cat. no. 12-00302/5/6/3
6) *Slimsub (EcoSep)	Gel size - 3 (w) X 10 (l) Cat. no. E2100031/32
7) *Ecosub (EcoSep)	Gel size - 7 (w) X 10 (l) Cat. no. E2200071/72
8) Midisub (EcoSep)	Gel size - 10 (w) X 10 (l) Cat. no. E2400101/102
9) Tubbysub (EcoSep)	Gel size - 15 (w) X 10 (l) Cat. no. E2300151/152

Preparation instructions:

0.5X tank buffer:

0.5X tank buffer is to be prepared from 5X tank buffer by diluting with D/W. Refer to the table below

Table 1:

	1 Expt.	5 Expts.	10 Expts.
D/W	270 mL	1350 mL	2700 mL
5X Tank buffer	30 mL	150 mL	300 mL
Total	300 mL	1500 mL	3000 mL

Store 0.5X tank buffer at 4°C.

Choice between TAE and TBE

The choice between TAE and TBE can be made based on the following properties
a) TAE has low buffering capacity and will get exhausted if electrophoresis is carried out for prolonged times.
b) TBE is more expensive than TAE.
c) Double stranded DNA fragments migrate faster through TAE than through TBE.
d) The resolving power of TAE is better for higher molecular weight and for supercoiled DNA. TBE is better for lower-molecular weight DNA.

Choice of Agarose concentrations

Agarose conc in gel (% w/v)	Range of separation of linear DNA	V grad-ient (V/cm)	Notes
0.3	5-60	5	Very fragile. Opt for PAGE if possible
0.6	1-20	5	
0.7	0.8-10	5	
0.8	0.5-7	10	BPB runs at approx. 1 kb, XC at approx. 4 kb
0.9	0.5-7	10	
1.0	0.5-6	10	
1.2	0.4-6	10	
1.5	0.2-3	10	BPB runs at approx. 0.5 kb
2.0	0.1-2	20	Pour agarose at 55°C in pre warmed tray
3.0	0.1-1	20	

Protocol summary:

1)Clean and dry the apparatus.
2)Prepare 0.5X tank buffer and chill in refrigerator.
3)Seal the sides of the tray with tape.
4)Place the comb in tray.
5)Melt agarose in hot water bath.
6)Cool to approximately 50°C and add EtBr (conc. 0.5 µg/mL), if desired.
7)Cast the gel and allow to set for 15-20 mins.
8)Remove the tape and place the tray in apparatus.
9)Pour chilled 0.5X tank buffer into the apparatus such that buffer should be 2-4 mm above the gel.
10)Remove the comb gently.
11)Load the sample and connect the apparatus to power supply.
12)Start the power supply. Adjust the voltage and current as per your requirement.
13)Switch off the power supply when the blue tracking dye reaches towards the end of the gel.
14)Visualize the bands under UV transilluminator.

Troubleshooting Guide

Indication	Cause	Remedy
1. Problems with power supply		
Power supply will not switch 'ON'. i.e. light on mains switch/LED indicator does not come 'ON'.	Check 230 V mains supply	Restore the mains supply
	Power supply fuse blown	Try replacing with correct fuse only once
	Fuse blows again	Contact the manufacturer of the power supply
2. Problems with horizontal electrophoresis unit		
Power supply 'ON' but no movement of samples/ bubbles in the buffer	Cathode and anode are not connected to the right output terminals.	Connect the cathode and anode to the right output terminals i.e. red to red and black to black
	Electrical connecting cords are not properly inserted.	Insert the electrical connecting cords properly.
	The connecting cords are damaged.	Use new connecting cords
	Low level of buffer in the apparatus	Raise the level of buffer in the apparatus such that the circuit is formed.
	Presence of cello tape in tray	Remove cello tape and check
	Oxidation/deposit formation on the contact terminals/ connectors	Disconnect the instrument. Clean the terminals. Reconnect the instrument and try again.
No bands seen	DNA was electrophoresed off the gel	Electrophorese the gel for less time, at lower voltage
	To low concentration of sample	Concentrate the sample before loading
	sample was degraded	Avoid nuclease contamination
	For ethidium bromide-stained DNA, improper UV light source was used.	Use short-wavelength (254 nm) UV light for greater sensitivity.

Separation of DNA and RNA by Agarose Gel Electrophoresis

Theory:

Electrophoresis is a technique in which charged molecules migrate from cathode to anode under influence of electric current. Agarose is a polysaccharide obtained from red algae. Agarose forms inert matrix through which charged molecules migrate towards anode. Smaller molecules move faster compared to larger ones, in a process called sieving. Higher the percentage of agarose lower is the pore size and vice versa. Most agarose gels are made between 0.7% and 2% of agarose depending on size of DNA/RNA to be separated. The DNA/RNA can be visualized by adding ethidium bromide (EtBr) in the gel or loading buffer.

Various factors can affect the migration of nucleic acids during electrophoresis: the percentage of agarose, ionic strength of the buffer, the voltage applied, the concentration of EtBr and size of nucleic acid being electrophoresed.

Detailed Protocol

1) Rinse all items, beakers, measuring cylinders, pipettes and electrophoretic apparatus with distilled water, dry and use.
2) Prepare 0.5 X tank buffer as mentioned in table 1. Cover with a plastic sheet and chill in the refrigerator.

Sealing the tray:

3) Tape both the ends of the tray making sure that the tape is high enough and place it on a levelled surface.

Casting the gel:

4) If using the comb stand: place the comb stand on the tray. If using the fixed height comb: insert the comb in the slots provided.

5) Melt the agarose in a hot water bath.

6) Cool the agarose to approximately 50°C and add EtBr (final concentration 0.5 µg/ml) if desired. Pour the warm solution into the tray to a gel thickness of about 4-5 mm. Allow the gel to set for minimum 15 mins.

7) Carefully remove the tape and place the gel tray in the apparatus such that the wells are closer to the cathode (black connector).

8) Pour 0.5X tank buffer into the apparatus such that buffer should be 2-4 mm above the gel.

9) Remove the comb gently.

Sample preparation:

10) Mix sample and loading dye in a proportion of 1:5 (i.e. for 5 µL of sample add 1 µL of dye)

Running the gel:

11) Load 20 µL of the sample per well.

12) Connect the apparatus to power supply. Start the power supply. Adjust the voltage and current as per your requirement. (For a small apparatus of 3 x 10 cm adjust to a constant voltage of 50-70) *Check if the samples are migrating in the correct direction.*

13) Switch the power supply off when the blue tracking dye reaches towards the end of the gel.

14) Remove the gel tray from the apparatus, drain out all the buffer carefully and dry the bottom surface of the gel tray with blotting paper. If the tray is UV transparent, place the gel tray directly over the window of trans-illuminator. If the tray is UV opaque, then remove the gel from the tray and place over the window of trans-illuminator/ gel

documentation system.

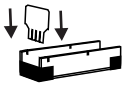
15) Switch on the trans-illuminator and visualize the bands.

16) Discard all buffers containing the nucleic acid intercalating dye into a beaker containing purple crystals of dye oxidiser.

Flow chart

1) Sealing of the tray

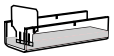
2) Place comb



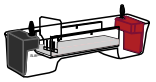
3) Melt agarose

4) Pour agarose into the tray

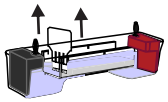
5) Remove the cello tape



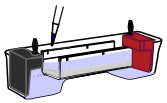
6) Place in tank



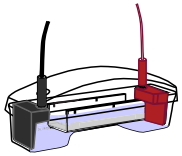
7) Pour buffer & remove comb



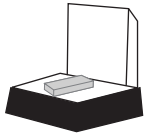
8) Load samples



10) Cover with lid & connect to PS*



11) Visualize the bands under UV transilluminator



PS*- Power supply