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# INSTRUCTION MANUAL OF DNA/RNA Isolation KIT

**(KT-NA001/ KT-NA005/ KT-NA010)**


**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 dissolution dye with EtBr | 4°C |
| 4. | EtBr (10 mg/mL) | 4°C |
| 5. | KMnO4 (Powder) | RT |
| 6. | D/W | RT |
| 7. | Homogenization buffer | RT |
| 8. | Sodium acetate (Precipitation buffer) | RT |
| 9. | TE buffer | RT |
| 10. | Chaotropic buffer | RT |
| 11. | Chloroform | 4°C |
| 12. | Ethanol | RT |
| 13. | 70% Ethanol | RT |

## Equipments and consumables required but not provided in the kit:

-Fresh Chicken brain (1g for 1 Expt.)
-Mortar & pestle
-Nylon filter pouch
-Ice bath
-Hot water bath
-Refrigerator
-Beakers (5, 15 mL): 1 no. each
-Centrifuge (for microfuge tube)
-Centrifuge tubes
-Horizontal gel electrophoresis apparatus [maximum gel size 7 (w) X 10 (l) cm]
-One 100 mL measuring cylinder
-Two 250 mL beakers
-Power supply
-UV 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 | |

## Troubleshooting Guide 1

| Indication | Cause | Remedy |
|---|---|---|
| No bands/very faint bands of DNA | Less tissue taken | Use more tissue for extraction |
| | Incomplete homogenization | Homogenize tissue thoroughly in homogenization buffer |
| | Heating not enough or overheated the homogenized tissue | Heat the homogenized tissue at proper temperature as mentioned in the protocol |
| | Poor EtBr staining | Use proper concentration of EtBr (i.e. 0.5 µg/mL) |
| Bands are fuzzy | Too much tissue was taken | Weigh 1 g of tissue as mentioned in the protocol |
| | DNA/RNA contaminated with proteins | Add appropriate amount of chloroform and spun vigorously |
| No bands/very faint bands of RNA | Nuclease contamination | Make sure all the glassware, plastic-ware and solutions are autoclave before use |
| | Incomplete homogenization | Homogenize tissue thoroughly in homogenization buffer |
| | Poor EtBr staning | Use proper concentration of EtBr (i.e. 0.5 µg/mL) |
| | Incomplete ethanol evaporation | Allow the ethanol to evaporate completely from RNA pellet before dissolving in TE buffer |

## Troubleshooting Guide 2

| 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 main 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. |

# DNA/RNA isolation and Separation of DNA/RNA by Agarose Gel Electrophoresis

## Introduction:

The isolation of DNA from any source usually begins with lysis, or breakdown of tissue or cells. Lysis is carried out in the salt solution containing detergent such as SDS to denature proteins. Lysis of the tissue is a crucial step which allows nucleic acids to be released from the nucleus by disintegrating it. Heating (at 50-60°C) during lysis step enhances the process and results in breakdown of cells. Once the lysis is complete, the lysate is treated with chloroform to remove all the proteins and lipids from the lysate. DNA is recovered from the aqueous solution by treating with ethanol. The isolation of RNA involves four basic steps. First, lysis of the cells by addition of lysis solution containing detergent. After cell has lysed, treat the lysate with chloroform to separate

RNA from the solution. RNA is recovered from the aqueous solution by addition of cold ethanol. Precipitated RNA is then washed with 70% ethanol to remove any salt or impurities from the solution.

## 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.

## DNA isolation protocol

1) Sterilize all the glassware by autoclaving.

2) Weigh 1 g of fresh chicken brain tissue.

3) Add 1.25 mL homogenization buffer and 190 µL Chaotropic buffer. Homogenize the chicken using mortar and pestle.

4) Transfer the paste in a small (5 mL) beaker. Heat at 50-60°C in water bath for 10 mins.

5) Add equal volume of chloroform (i.e. 1.5 mL) and mix well.

6) Transfer the content in a centrifuge tube and spin hard for 10-15 mins.

7) Collect supernatant in a chilled beaker (15 mL) or tube and cool in freezer/keep on ice bath for 10 mins.

8) Add 2 ½ volume of ethanol (i.e if volume is 3 mL then add 7.5 mL of ethanol) to the supernatant from sides of the beaker/tube very slowly.

9) Swirl gently and cool in freezer/keep on ice bath for 10 mins.

10) Collect DNA fibers in a microfuge tube with a glass rod. Again swirl the content gently and keep in freezer/ ice bath. Collect the DNA fibers in a same microfuge tube.

11) Add 0.5 mL TE buffer and store at 4°C.

## RNA isolation protocol

1) Sterilize all the glassware by autoclaving.

2) Weigh 1 g of fresh chicken brain tissue and place tissue in nylon filter pouch.

3) Immerse the bag of tissue in a mortar containing 2 mL homogenization buffer.

4) Mash the tissue by crushing it within bag. Empty bag contents in mortar.

5) Add 300 µL precipitation buffer, mix and add equal volume of chloroform. Mix vigorously.

6) Transfer the content in sterile microfuge tube and spin hard for 15 mins.

7) Remove the supernatant in sterile microfuge tube. Again spin to remove debris and fibres.

8) Collect the supernatant in tube and add 2.5 mL cold ethanol. Mix thoroughly and chill for 10 mins. on ice bath.

9) Spin to collect the precipitate.

10) Wash the precipitate with 70% ethanol. Spin for 15 mins.

11) Discard the supernatant and keep the tube open at RT until the ethanol has evaporated.

12) Re-suspend the precipitate in 0.5 mL TE. Store at 4°C.

## Electrophoresis 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.