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INSTRUCTION MANUAL OF PAGE KIT

(KT-PG001/ KT-PG005/ KT-PG010)

Manufactured and Marketed by:

TechResource

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Kit components

Sr. no.	Components	Store at
1.	5X tank buffer for PAGE	RT
2.	Quick stain solution	RT
3.	Fixing solution	RT
4.	Sealing agar	RT
5.	Sample loading dye	4°C
6.	Well filler solution	4°C
7.	Separating buffer	4°C
8.	Stacking buffer	4°C
9.	Acrylamide	4°C
10.	TEMED	4°C
11.	APS (powder)	4°C
12.	D/W	RT

Equipments and consumables required but not provided in the kit:

- Vertical Electrophoresis unit (maximum size 7 x 8.5 cm)
- Power supply
- Glass pipette (1, 5 and 10 mL) 1 each
- Small beakers (250 mL) 2 no.
- Tube float
- Measuring cylinder
- Centrifuge (for microfuge tube)
- Hot water bath
- Refrigerator
- Forceps
- Micropipettes:
  - 0.5-10 µL
  - 5-50 µL
  - 10-100 µL
  - 100-1000 µL
- Micropipette tips

Vertical electrophoresis apparatus

We manufactured electrophoresis apparatus, those marked as * are suitable for this kit.

1)*Microkin Gel size - 8.5 (w) X 7.5 (l) cm
Cat. no. 02-00142/144

2) Monokin Gel size - 8.5 (w) X 12 (l) cm
Cat. no. 03-00184/182

3) Midikin Gel size - 16.5 (w) X 16 (l) cm
Cat. no. 05-00162

4) Macrokin-S Gel size - 18.5 (w) X 18 (l) cm
Cat. no. 04-00192/193

5) Macrokin Gel size - 18.5 (w) X 24 (l) cm
Cat. no. 04-00312/313

6) Megakin Gel size - 30 (w) X 30 (l) cm
Cat. no.

7) Minikin Gel size - 10 (w) X 12 (l) cm
Cat. no. 01-00000/01

8)*Slimpage Gel size - 4 (w) X 8.5 (l) cm
Cat. no. E0100142

9)*Ecopage Gel size - 7.5 (w) X 8.5 (l) cm
Cat. no. E0200142

10) Tubbypage Gel size - 16 (w) x 14 (l) cm
Cat. no. E0300142

Preparation instructions:

Table 1: For separating gel (10%)

Chemicals	For Slimpage (Gel size- 4X8.5 cm)	For Microkin/Ecopage (Gel size- 8.5X7.5)
D/W	2.4 mL	4.5 mL
Separating buffer	1.52 mL	2.78 mL
30% Acrylamide soln.	2.0 mL	3.7 mL
10% APS soln.	30 µL	60 µL
TEMED	4 µL	7 µL
Total	6 mL	11 mL

Note: Add TEMED and APS at the time of pouring the solution in the sandwich since polymerization starts immediately on addition of these chemicals

Table 2: For stacking Gel (4%)

Chemicals	For Slimpage (Gel size- 4X8.5 cm)	For Microkin/Ecopage (Gel size- 8.5X7.5)
D/W	1.2 mL	2.4 mL
Stacking buffer	0.5 mL	1 mL
30% Acrylamide soln.	0.27 mL	0.54 mL
10% APS soln.	10 µL	20 µL
TEMED	2 µL	5 µL
Total	2 mL	4 mL

Note: Add TEMED and APS at the time of pouring the solution in the sandwich since polymerization starts immediately on addition of these chemicals

1X tank buffer:

1X tank buffer is to be prepared from 5X tank buffer by diluting with D/W.

Table 3:

	1 Expt.	5 Expts.	10 Expts.
D/W	120 mL	600 mL	1200 mL
5X Tank buffer	30 mL	150 mL	300 mL
Total	150 mL	750 mL	1500 mL

Store 1X tank buffer at 4°C.

10% APS:

In 1.5 mL tube, weigh 0.1 g of APS from a microfuge tube labelled as APS and add 1 mL D/W. Mix to dissolve and store in dark at 4 °C. (*Prepare just before use*)

Protocol summary:

1) Clean and dry the apparatus.
2) Assemble the unit and seal with agarose.
3) Place the comb and mark the level till which the separating gel needs to be poured.
4) Prepare separating gel as per table no.1
5) Pour the separating gel till the level marked in above step and overlay with D/W.
6) Allow to polymerize for 30-45 mins.
7) Prepare stacking gel as per table 2.
8) Flick off the overlay D/W.
9) Pour the stacking gel and immediately insert the comb.
10) Allow to polymerize for 10-15 mins.
11) Mark the wells and Remove the comb gently.
12) Fill the apparatus with chilled 1X tank buffer.
13) Prepare the serum sample.
14) Load the samples. Connect to a power supply and carry out electrophoresis at required Voltage.
15) Switch the power supply off when the blue tracking dye reaches the bottom of the gel.
16) Remove the gel from the assembly and stain with staining solution.

(Refer to detailed protocol on the reverse)

Separation of serum proteins by polyacrylamide gel electrophoresis and staining the protein bands

Introduction:

Polyacrylamide gel electrophoresis (PAGE) is a commonly used technique to separate proteins and small nucleic acids on the basis of their size, shape and/ or charge.
Serum contains over one hundred proteins, each having a specific set of functions. Among these, albumins and globulins are the major proteins in serum and can be separated into five fractions using electrophoresis; albumin, alpha 1 globulin, alpha 2 globulin, beta globulin and gamma globulin.
Serum electrophoresis band patterns are used in disease diagnostics as being indicative of disease state. Separated serum proteins are stained by performing Coomassie brilliant blue staining.

Theory:

A solution of acrylamide and bisacrylamide is polymerized to from polyacrylamide gel. Acrylamide alone forms linear polymers. The bisacrylamide forms crosslinks between polyacrylamide chains. The gel pore size depends on the concentration of acrylamide, and the ratio of acrylamide to bisacrylamide. Higher the concentration of acrylamide smaller the pore size. Polymerization of acrylamide and bisacrylamide is induced by addition of ammonium persulphate (APS), which spontaneously decomposes to form free radicals. TEMED is a free radical stabilizer which improves the acrylamide polymerization. Sodium dodecyl sulphate (SDS) is an amphipathic detergent. SDS binds non-covalently to proteins and cause denaturation. SDS-treated proteins have similar charge-to-mass ratio and hence its rate of migration is completely based on its molecular weight. SDS PAGE system is a discontinuous gel with an upper stacking gel and lower resolving gel that have different poly-acrylamide concentrations and pH values. The stacking gel has a lower percentage of polyacrylamide allowing proteins to move quickly and stack into a tight band before entering resolving gel (higher percentage of polyacrylamide) for separation.

Detailed Protocol

Assembling and sealing the apparatus:

1) Rinse all items, beakers, measuring cylinders, pipettes, funnels and electrophoretic apparatus with distilled water (As the chlorine in tap water will adversely affect the stacking); dry and use .

2) Prepare 1X tank buffer as mentioned in table 3. Cover with a plastic sheet and chill in the refrigerator.

3) Assemble the PAGE apparatus (As described in the instrument manual).

4) If you are using agarose free gel casting system for Microkin then skip to next step, else, melt the sealing agar in a hot water bath. Let it cool to about 45 °C. Seal the bottom of the sandwich by pouring 5 mL of the molten agar into the trough in the lower tank, also seal the slot of the spacer with hot agar. Let the agar set for 10 mins.

Casting the Gel:

5) Place the comb in assembled apparatus. Using a permanent marker, mark the level till which the separating gel needs to be poured. (This can be about 1 cm below the level of the bottom of the wells of the comb). Remove the comb from the apparatus.

6) In a clean beaker add the components of the separating gel as per Table 1. (Add TEMED and APS at the time of pouring the solution in the sandwich since polymerization starts immediately on addition of these chemicals.)

7) Use a beaker or a pipette to pour the separating gel solution in the assembled cassette till the level marked.

8) Using a 1 mL pipette carefully overlay D/W over the separating gel. This is done to prevent oxidation of the separating gel buffer which may result in incomplete polymerization.
Do not use butanol as it damages the apparatus.

9) Let the gel polymerize for 30-45 mins. Do not shift the apparatus.

10) Flick off the overlay water, blot out any remaining water with filter paper making sure no lint is left behind The water remaining behind could dilute out the stacking gel that is being poured.

11) In a clean beaker add the components of the stacking gel as per table 2. Use a pipette to pour the stacking gel and immediately insert the comb.

12) Make sure no air bubbles are trapped in between the wells. If there are any air bubbles remove the comb halfway and insert again.

13) Mark the position of the wells with a permanent marker.

14) Let the gel polymerize for 10-15 mins. (meanwhile you can skip ahead to the sample preparation step to save time)

15) At the end of the period for polymerization slowly pull out the comb ensuring that the wells don't collapse.

16)Wash the wells with D/W to remove any unpolymerized acrylamide.

17) Fill the cathodic and anodic chamber with chilled 1X tank buffer and fill the wells with well filler solution.

Sample Preparation:

18) Add 20 µL of serum to 20 µL of sample loading buffer (1:1). Mix,.centrifuge momentarily to collect the
mixture at the bottom of the tube.

19) To denature the serum, place the tube in a float in a hot water bath at 70°C for 5 mins. or at 100°C for 1 mins.

20) Remove the float from the water bath with forceps and place the tubes in a refrigerator / chilled water to cool quickly. Once cooled, centrifuge the tubes for 10 min in a centrifuge at 5,000 rpm.

Running the gel:

21) Load 20 µL of the centrifuged denatured sample (serum-buffer mixture) in each well.

22) Connect the apparatus to power supply. Start the power supply. Adjust the voltage and current as per your requirement. (For a small apparatus of 8.5 x 7 cm adjust to a constant voltage of 100 V)

23) Switch the power supply off when the blue tracking dye reaches the bottom of the gel.

24) Remove the sandwich from the apparatus and carefully prise open the sandwich. Mark the orientation of the gel by cutting off a small triangular portion from the lower left-hand corner of the gel. If you are using glass plates and not the fibre glass plate provided with the Microkin, then skip to step 25.

25) The polyacrylamide gel attaches itself firmly to the fibre-glass plate. To peel gel from this plate, insert the gel-detacher under the gel, and while doing so pour water along its edge. Slide the gel-detacher towards the opposite edge, and pour water behind the gel-detacher to ensure that the gel does not re-adhere to the plate.

Staining the gel:

26) Slide the gel into the staining tray and pour 15 ml of fixing solution over it. Place the tray on a rocker or shake the tray periodically. After 10 mins., discard the solution and add 15 mL fresh fixing solution. Shake the tray periodically. After 10 mins., discard the solution. It is important to drain out all the fixing solution before adding the staining solution. Prevent the gel from slipping out of the tray.

27) Add 15 mL of staining solution and place the tray on the rocker or keep shaking the tray continuously with hand for 5 mins. (if shaking by hand then after 5 mins. the tray can be shaken periodically). Bands will start to develop in about 20 mins.

28) Observe against a white background. With the passage of time, additional bands will continue to appear and existing bands will get more intense.

29) When the staining has been developed to your satisfaction, remove the staining solution and add D/W to wash the gel. Hold the gel against white background to observe stained bands.