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**INSTRUCTION MANUAL
OF**

**HORIZONTAL GEL ELECTROPHORESIS
APPARATUS**

GREYWHALE/ GREYWHALE -R/ GREYWHALE -RC

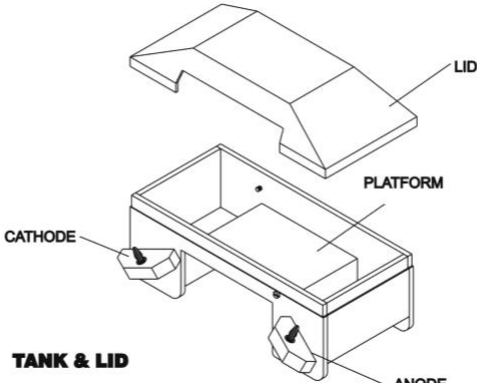
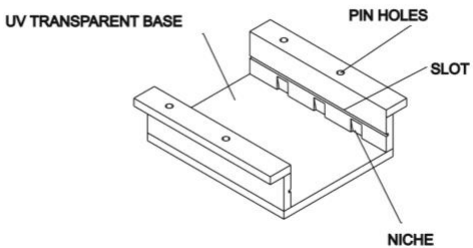
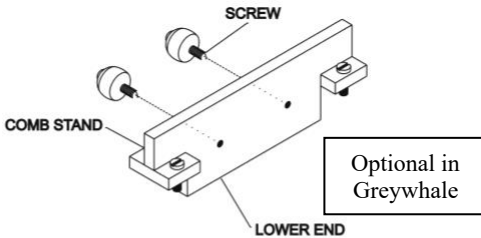
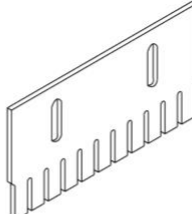
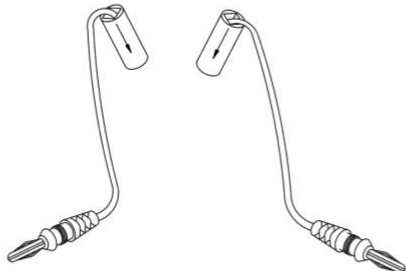
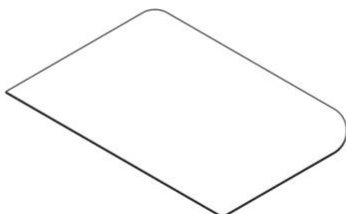
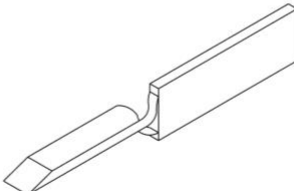
(NEW CAT.No THGWR-003030/ THGWR-R03030/ THGWR-RC3030)

(OLD CAT. No. 12-00302/ 12-00305/ 12-00306)

Greywhale – *For DNA and protein separation*
Greywhale-R – *For RNA, DNA and protein separation*
Greywhale-RC – *For RNA, DNA and protein separation,
with cooling and buffer circulation*

TechResource
Scientific Pvt Ltd

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Essential components of the Apparatus:	
1. Tank with male connectors and lid for safety	2. One UV-transparent tray with gel gripping niches and precisely positioned holes
3. Fixed height combs (54 well); 1.5 mm thick.	4. One set of connecting cords
Additional handy components provided:	
1. One gel slicer	2. One niche cleaner
1  TANK & LID	2  TRAY
3  COMB STAND	4  PMMA COMB
5  CORDS	
6  GEL SLICER (ACCESSORY)	7  NICHE CLEANER (ACCESSORY)

The line diagrams are only for representational purpose. The actual product may vary in terms of size and detail.

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Additional accessories that can be ordered for convenience:(For full list visit the site www.technosource.in)**Trays**

Gel width	Gel length	Opaque/UVT	Gel gripping	Cat. No.	New Cat.No.
30 cm	5 cm	UV-Opaque	No	12-02051	THGWS-T03005
30 cm	5 cm	UVT	Yes	12-02052	THGWS-TU3005
30 cm	10 cm	UV - Opaque	No	12-02101	THGWS-T03010
30 cm	10 cm	UVT	Yes	12-02102	THGWS-TU3010
30 cm	20 cm	UV - Opaque	No	12-02201	THGWS-T03020
30 cm	20 cm	UVT	Yes	12-02202	THGWS-TU3020
30 cm	25 cm	UV - Opaque	No	12-02251	THGWS-T03025
30 cm	25 cm	UVT	Yes	12-02252	THGWS-TU3025

Combs

For tray of width (in cm)	No of wells	Comb thickness (in mm)	Cat. No.	New Cat.No.
30	33	1, 1.5, 2	12-09331/2/3	THGWS-CML331 THGWS-CML332 THGWS-CML333
30	54	1, 1.5, 2	12-09541/2/3	THGWS-CML541 THGWS-CML542 THGWS-CML543
30	65	1, 1.5, 2	12-09651/2/3	THGWS-CML651 THGWS-CML652 THGWS-CML653
30	92	1, 1.5, 2	12-09921/2/3	THGWS-CML921 THGWS-CML922 THGWS-CML923

Others

Name and description	Cat no	New Cat. No.
Gel casting chamber for tray 30 cm (w) and varying length upto 30 cm	12-25302	THGWS-CC3030
Gel slicer, breakage resistant	63-05300	THC0S-GS2930
Niche cleaner	64-06075	THC0S-NC0001
Penta legged levelling table 38 x 52 with spirit level	69-41529	TCC0S-LT3852
Ligogel Tray and comb	65-08539 & 65-05042	THLGS-LG0001 THLGS-CM0405

Caution

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Use the apparatus with care. It should be washed and rinsed with soft water, air dried at room temperature and stored covered.

It should not be

- Subjected to impact
- Exposed continuously to temperatures outside the range of 0-40°C
- Handled with abrasive or sharp objects,
- Exposed to organic solvents such as aromatic or halogenated hydrocarbons, ketones, esters, alcohols over 30% or concentrated acids over 25%,
- Exposed to other solutions which will attack plastics such as acrylic, nylon and polycarbonate.

To avoid electrolytic corrosion always dry the connecting pins on central electrode unit before attaching the electrical cords to them.

Other items needed to perform horizontal gel electrophoresis

1. To clean the apparatus
 - a. Abrasive and enzyme free detergent
 - b. De-mineralized tap water
 - c. Squirt bottle containing distilled water
2. To assemble the apparatus
 - a. Tissue paper
 - b. Blotting paper
 - c. Sealing tape/ gel casting stand
 - d. A pair of scissors
3. To prepare the agarose gels
 - a. Stock buffer
 - b. Agarose powder
 - c. Pipettes (10 ml and 5 ml)
- d. Ethidium bromide (EtBr) [optional]
- e. Micropipettes
- f. Leveling table [optional]
- g. Magnetic stirrer with hot plate/ microwave oven
4. To prepare and load the samples
 - a. Polypropylene tubes,
 - b. Dye (bromophenol blue) in buffer
 - c. High speed centrifuge
 - d. Micropipettes
5. To separate the sample components
 - a. A constant voltage power supply with minimum rating of 200 V, 200 mA

Assembling the apparatus

1. Place the comb in position on the tray
2. Seal the sides of the tray with the tape [or clamp the tray in the casting stand]
3. Place tray on a leveled surface and pour the agarose solution (at about 50°C) into the tray
4. Allow to cool for 15 mins
5. Remove the tape and comb [or remove from casting stand]
6. Place the tray in the tank
7. Pour buffer into the tank such that the gel is just submerged (about 2000 mL)
8. Load the samples with a micropipette
9. Cover the tank, connect to a power supply and carry out electrophoresis at 120-150 V
10. Stop the power supply when the dye front reaches the end and disconnect the apparatus
11. Remove the tray and place it directly on the trans-illuminator to view

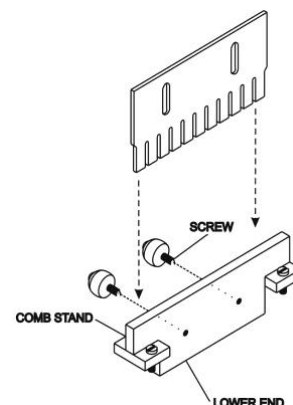
Detailed procedure for assembling the apparatus

I. Cleaning the apparatus

1. Rinse the tank thoroughly with water. In case the tank is dirty, clean it carefully with a dilute soap solution, wash thoroughly with water and finally rinse with distilled water. (As EtBr is present it is advisable to use gloves while cleaning). Invert to dry.
2. Exert utmost care while cleaning, rinsing and drying the UV-transparent tray to avoid scratching the UV-transparent surface. Stand the tray on its edge to dry.
3. Wash the comb and comb-stand as in I.1.

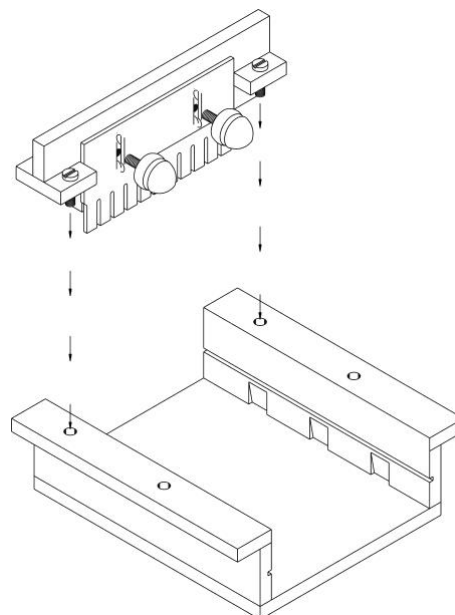
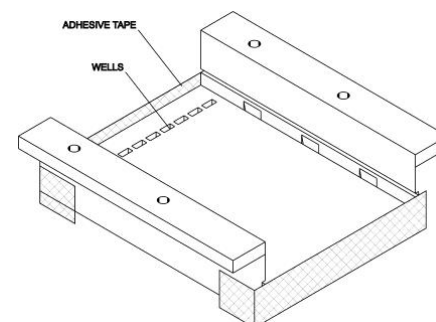
II. Adjusting comb height (If you are using fixed height combs go to step III)

1. The comb stand allows you to place the comb anywhere along the length of the tray when the pins on the comb stand are raised
2. To adjust the comb height, mount the comb on the comb stand. Place the comb stand on the tray [the pins fit into the holes on the tray handles].
3. Place a strip of plastic or card paper of uniform thickness (about 1 mm) under the teeth of the comb. Loosening the screws on the comb stand allow the teeth of the comb to rest on the strip. Tighten the screws. Raise the comb-stand and remove the flat object.



III. Sealing the tray

1. Sealing the tray with tape (If you have a gel casting stand go to step III.2)
 - i. To prepare the UV-transparent tray for gel casting; cut two 36 cm strips of tape. [1/2" wide electrical insulation tape is well suited to contain the amount of agarose solution poured].
 - ii. Tape both the ends of the tray making sure that the tape is high enough
 - iii. Place the tray on a smooth, leveled surface. Ensure that the tape does not protrude below the bottom of the tray else agarose will set at a slant.
 - iv. There are no crinkles along the tape, through which agarose solution could leak.
2. Using the casting stand (if you have sealed the tray with the tape go to step IV)
 - i. On the gel casting stand turn the clamping screw anti-clockwise and push the clamp back.
 - ii. Remove the positioning screws by turning them anti-clockwise and place the moving gasketed dam such that the tray fits in between the rubber gaskets.
 - iii. Shift the moving gasketed dam away from the tray till the holes of the screws align with any of the holes in the base.
 - iv. Tighten the positioning screws to keep the moving gasket in place.

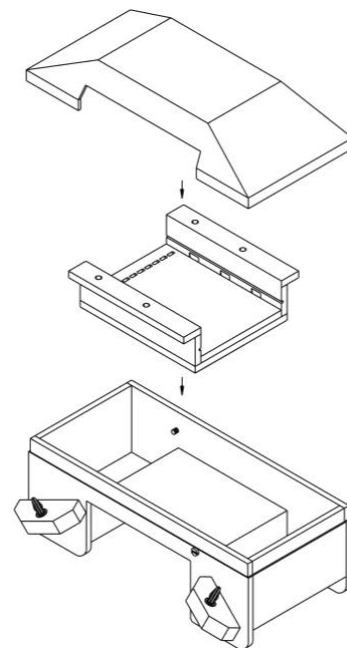


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- v. Place the tray in the stand and tighten both the clamping screws clockwise to seal the edges of the tray. DO NOT over tighten.

IV. Casting the gel

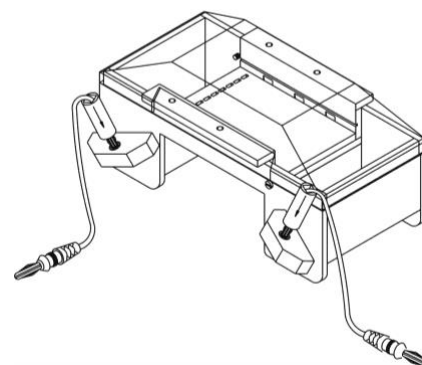
1. If using the comb stand: place the comb stand on the tray.
If using the fixed height comb: insert the comb in the slots provided.
2. Ensure that the agarose solution is at 50°C and add EtBr (final concentration 0.5 µg/ml) if desired. Pour the warm solution into the tray to a gel thickness of about 4-5 mm. Allow the gel to set for minimum 15 mins.
3. Pour some buffer over the comb and carefully lift the comb stand [lift the comb if using the fixed height comb]. Wash the comb and keep it aside.
4. If you are using the casting stand; loosen the clamping screw (turn it anti-clockwise) remove the tray carefully from in between the gaskets. If you are using tape to seal the ends then drain the buffer into the tank and peel the tape from the ends.
5. Place tray on the platform such that the wells are nearer the (black) cathode. The wells may be cleaned by gently flushing them with buffer.



V. Loading the samples and performing gel electrophoresis

1. Pour buffer into the tank to a level of 1-2 mm above the gel surface (Refer table for volumes).
2. Load the samples containing glycerol and appropriate tracking dyes in the wells.
3. Cover the tank with the lid
4. Connect the electrical cords (red to red and black to black)
5. Run the gel at constant current or constant voltage (a constant voltage of 120 V -150 V is preferred).
6. When the dye front reaches the end of the gel, electrophoresis is complete.

Note: in case you want to perform the electrophoresis for a longer time; continue the run even after the dye front has moved out of the gel. For a shorter run discontinue after the dye reaches half way.



VI. Dismantling the apparatus:

1. After the run is complete, disconnect the electrical cords and remove the lid.
2. Lift the tray carefully at an angle to drain the buffer, blot off the excess buffer from the base of the tray and place it on a trans-illuminator/ gel documentation system. (The tray is provided with handles to make this task easier, and to prevent any contact with the buffer containing EtBr. Please wear gloves to ensure safety.)
3. Use the gel slicer to cut along the sides of the gel and gently slip it out of the tray.
4. Remove the gel from the niches with the help of the niche cleaner. (EtBr is a potent carcinogen, therefore discard it appropriately.)
5. In case you wish to use the gel later, tape up both the sides of the tray put in a little buffer, put it in a sealable bag and store it away at 4°C.
6. Store the electrophoresis tank buffer in a container, it can be reused for 2-3 occasions (Note: the buffer contains some amount of EtBr if it was used in the gel)
7. Wash and dry all the parts.

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Tips and notes

1. Preparing agarose: In a clean, dry conical flask add agarose powder (low electro-osmosis grade) followed by appropriate amount of stock buffer, and D/W. Introduce a Teflon coated magnetic spin bar into the flask, cover its mouth with foil and place it on a hot plate magnetic stirrer. While it is being heated stir at medium speed (avoid bubble formation). The solution should turn clear after which the magnet is removed, the solution is allowed to cool to the appropriate temperature (see notes in 6 below) and poured into the gel tray.
2. EtBr should be added only to the agarose solution which can be cooled to 50-55°C.
3. Storage of gelled agarose: Store the gelled agarose in air tight containers at 4°C in required aliquots. In case the agarose is repeatedly melted, its concentration will increase.
4. Reuse of cast gel: If the gel needs to be reused, connect the apparatus to a power supply (designed for safe overnight experiments) and run the gel overnight at a constant current of 10mA to migrate the DNA from the gel. Check that the gel is free of any DNA before re-using.
5. The migration of DNA in an electric field depends on a number of factors such as conformation (super-coiled, nicked or linear), composition of buffer (type, ionic strength, pH), presence of EtBr and concentration of agarose. In an electric field the DNA first orients itself in an end on position and then migrates at rates that are inversely proportional to the $\log_{10}$ number of base pairs. Larger molecules migrate slower than expected because of greater frictional drag and because they worm their way through the pores less efficiently than small molecules.
6. Guide in choosing gel concentrations for separating DNA.

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

7. Guide in choosing the amount of agarose for Greywhale/ Greywhale-R/ Greywhale-RC. Use the table below to obtain the amount of agarose (in g) to weigh for the required percentage of gel and gel volume for an approximate gel height of 5 mm

Tray size (cm)	Volume of gel (mL)	Agarose percentage							
		0.3%	0.6%	0.7%	0.8%	1%	1.2%	1.5%	2%
30 (w) x 5 (l)	40	0.23 g	0.45 g	0.53 g	0.60 g	0.75 g	0.90 g	1.13 g	1.50 g
30 (w) x 10 (l)	80	0.45 g	0.90 g	1.05 g	1.20 g	1.50 g	1.80 g	2.25 g	3.00 g
30 (w) x 15 (l)	120	0.68 g	1.35 g	1.58 g	1.80 g	2.25 g	2.70 g	3.38 g	4.50 g
30 (w) x 20 (l)	150	0.90 g	1.80 g	2.10 g	2.40 g	3.00 g	3.60 g	4.50 g	6.00 g
30 (w) x 25 (l)	375	1.13 g	2.25 g	2.63 g	3.00 g	3.75 g	4.50 g	5.63 g	7.50 g

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