Standard Operating Procedure: Electrophoresis Power Supply

Device Model: PNP-1000

Manufacturer: Padideh Nozhen (Made in Iran)

1. Purpose

To provide a standardized procedure for the operation and maintenance of the **PNP-1000 Electrophoresis Power Supply** to ensure accurate separation of nucleic acids.

2. Scope of Operation

Electrophoresis is primarily used for the separation of **DNA and RNA** fragments. The principle of separation relies on the migration of charged molecules through a matrix (agarose gel) under the influence of an electric field. The molecules move from the **negative electrode (cathode)** toward the **positive electrode (anode)** within a specific running buffer contained in the electrophoresis tank.

3. Responsibility

The responsibility for executing this protocol lies with the relevant laboratory experts and technicians.

4. Operating Procedure

A. Gel Preparation

1. **Agarose Casting:** Dissolve agarose powder in the required buffer by boiling until completely clear.
2. **Sealing:** Seal the gel tray/casting tray using waterproof tape to prevent leakage.
3. **Comb Placement:** Position the comb so that it sits approximately **1 mm** above the bottom of the tray, then pour the molten agarose into the tray.
4. **Comb Removal:** Once the gel has solidified, carefully remove the comb and the sealing tape from around the gel chamber.

B. Sample Loading and Setup

1. **Buffer Addition:** Fill the electrophoresis tank with **TBE buffer (0.5X - 1X)** until the buffer level is approximately **0.5 cm above** the surface of the gel.
2. **Loading Samples:** Load the samples into the gel wells along with an appropriate **molecular weight marker (DNA Ladder)**.
3. **Note on Loading Dye:** If the PCR product does not contain a pre-mixed dye, use a **6X Loading Dye** (which contains DNA density agents). Mix the sample and dye at a ratio of **5 parts PCR product to 1 part Loading Dye**.

C. Running the Electrophoresis

1. **Power On & Pre-setting:** Turn on the power switch located at the rear of the device. **Before connecting the cables to the tank**, adjust the voltage settings to between **90V and 110V**.
2. **Tank Connection:** Place the gel inside the tank and add the running buffer (ensuring it covers the gel by 0.5 cm). Close the tank lid securely.
3. **Electrode Connection:** Connect the electrodes to the tank.

- **Black Cable →\rightarrow→ Negative Pole (Cathode)**
- **Red Cable →\rightarrow→ Positive Pole (Anode)**

4. **Crucial Directional Note:** Because DNA is negatively charged, it migrates from the **Cathode (-)** toward the **Anode (+)**. Therefore, the wells must be positioned at the **Black (negative)** end of the tank to ensure the DNA runs toward the Red (positive) end.

D. Post-Run Procedures

1. **Power Down:** Once the electrophoresis is complete, turn off the power supply first. Disconnect the cables by **grasping the plug heads** rather than pulling the wires.
2. **Gel Retrieval:** Remove the tank lid and carefully transfer the gel to an imaging system (Gel Doc) for visualization.
3. **Cleaning:** Empty the buffer from the tank. Thoroughly rinse the tray, comb, and tank with **distilled water**.

Warning: Failure to promptly empty and clean the electrophoresis tank, or the continued use of old, precipitate-heavy buffers, will cause the internal connection wires to lose their conductivity over time.

5. Safety Instructions

1. **PPE:** Always wear appropriate laboratory gloves during operation.
2. **Stability:** Never attempt to move or reposition the power supply unit while it is switched ON.
3. **Voltage Control:** Avoid connecting the tank to voltages exceeding the recommended limits. Excessive voltage causes buffer heating, which degrades both the gel integrity and the sample quality.
4. **Electrical Hazard:** To prevent electric shock, **never** attempt to add or remove a gel from the tank while the power supply is connected and turned on.
5. **Lid Safety:** Always keep the tank lid closed during operation to prevent accidental contact with the electric field.
6. **Liquid Spills:** Prevent any liquid from spilling onto the power supply unit, as this can damage the electronic circuit boards and significantly increase the risk of electric shock.
7. **Contamination Control:** To minimize cross-contamination, do not move electrophoresis equipment and accessories to other departments within the laboratory.

6. Maintenance

1. **Conductivity Preservation:** As noted previously, timely drainage and cleaning of the tank are mandatory. Using aged or sediment-heavy buffers leads to the degradation of the tank's internal wiring and reduces conductivity.

2. **Cable Handling:** To prevent wire breakage or electrical interruptions, always grasp the **connector head** when plugging or unplugging cables from the tank.
3. **Drying:** At the end of every work session, rinse all utilized components (tank, tray, comb) with distilled water and ensure they are completely dried before storage.