

PhastSystem Guidelines

Running electrophoresis media:

SDS or native polyacrylamide gels:

gradient: 10-15% or 8-25%

homogeneous: 7.5%, 12.5% or 20%

Preparing the gel compartment:

- Switch the system on (button in the back of the system, right hand side) and set the standby temperature for the first step of the method you wish to run (keyboard: press set standby temp., cursor to temp. on screen, press CE to clear, enter temp. on number pad, press do.)
- Raise applicator arm, then raise electrode assembly. Adjust electrode assembly to highest position by pressing red eccentric levers (lower right and left of electrode assembly) back until they click into place.
- Wipe off separation bed with a moist, lint-free cloth to remove dust particles. Wipe off electrodes **GENTLY** with cloth that does not leave dust particles. Do not touch the electrodes with your fingers; as finger proteins may distort results.
- Place a drop of water (60-75 μ l) onto the middle of the gel area(s) outlined in red.
- Remove gel pack from the refrigerator, cut along three sides to open pack, and remove gel from package with forceps using the plastic tab (keep plastic protective film on gel until right before using). Place gel onto a clean, hard surface, and carefully bend up the plastic tab for easier positioning onto the gel bed. Use a waterproof pen to mark the underside of the gel if desired.
- Lower the gel onto the gel bed so that a film of liquid free of air bubbles forms between the gel support and the separation bed. Remove any bubbles by sliding the gel around. Finally, position the gel so that its edges are in perfect alignment with the red lines (with the tab towards cathode (-)).
- Remove excess liquid from the gel bed with absorbant paper. Use forceps to gently lift and peel plastic film from the gel surface.
- Place the buffer strip holder over the gels, using the two black pins and the holder holes to form a hinge. Lower the buffer strip holder onto the separation bed.
- Remove buffer strips from a pack with a spatula. Wear gloves when handling strips to avoid contamination with finger proteins. Insert the strips into the holder and press gently to ensure good contact between the buffer strips and the gel.
- Lower the electrode assembly so that the outer electrodes rest evenly on the buffer strips. Press down along the top of the electrodes; they must have complete contact with the buffer strips.

- Lower the sample applicator arm. Load the desired sample applicator as follows:
Make a loading chamber by placing a piece of parafilm paper side up over the sample-well stamp, then run a hard object along a lane of wells to make a depression on the film. Remove the protective cover, then fill the depressions with twice the applicator capillary volume. Mark the applicator if desired and slide the loaded sample applicator into the slot nearest the cathode (-). Take care not to touch the gel. Lower the separation compartment lid.

Starting the Run for Electrophoresis Media:

- Set the standby temperature if you have not already done so.
- Prepare the gel compartment.
- Press "SEP start/stop" and enter the number of gels for this run, then press do. Methods are programmed for one gel. If two gels are entered here, current and power will be adjusted so the both gels run under the same conditions according to the programmed methods.
- Enter the number of the method you plan to run, then press do.
- The method name (if given) will appear. Press do to confirm and begin run. Once the separation bed is at the proper temperature the running parameters will appear on the screen.
- To stop a method press "SEP start/stop", then press do to confirm. The display will show the temperature and the accumulated volthours of the method just ended. Proceed with development immediately.

Running a Developing Method:

- Prepare the development unit as follows:
 1. Remove the caps from the Cap Set from the ports you plan to use.
 2. Connect the ports (1-9) as required to the solution bottles with PVC tubing.
 3. Connect port 0 to waste: use an empty bottle.
 4. Check for kinks in the tubing and make sure that the tubing is securely submerged in the solutions. NOTE: The chamber fills with 70 ml of solution. The bottles should be filled with at least 75-80 ml of solution to allow for residual solution in the tubes.
 5. Open the lid of the development chamber by pressing the right end of the red bar.
 6. Check that the chamber gasket on the lid is secure.
- Inserting the gel(s):
 1. Remove one gel from the separation bed using forceps -- be careful not to touch the gel with your fingers.
 2. Slide the gel, gel surface DOWN, into the upper position of the gel holder. (If you are only running one gel, slide it into the lower position gel surface UP.) Slide a second gel into the lower position, gel surface UP.

3. Close the lid by pressing down on the top of the lid and pushing in the red bar simultaneously

· Starting the Run:

1. Press "DEV start/stop" and enter the programmed method number.
2. Method name (if given) will appear. Press do to confirm and begin run.

· To stop a run temporarily:

1. Press "DEV pause/continue". The DEV on LED will blink and an alarm will sound at twenty second intervals until the run is continued. Press the button again to continue.

· To terminate a run:

1. Press "DEV stop/start" and then press do to confirm. The development chamber will empty and the in port tube is cleared.
2. Run a cleaning method by leading the tubes (1-9) into a bottle containing at least 700ml of distilled water. Lead tube 0 to waste: use and empty bottle. Open the lid of the chamber, check that the gasket is secure, and insert the Level Sensor Shield if it is not already there. Close the lid and press "DEV start/stop" then select cleaning method number (9). Press do to confirm and begin the method.

PhastSystem Maintenance:

· Electrodes should be cleaned after each use to prevent agarose/polyacrylamide buildup. The best way is to remove the assembly from the unit, gently brush the electrodes with a soft toothbrush, and rinse under running water. DO NOT soak the electrodes or touch them with your fingers. Allow assembly to dry after rinsing.

· Always run a cleaning cycle after staining is completed. Gently clean the level sensor shield with cotton tip applicator to remove residue, especially after silver staining. Visually inspect the lid gasket and tubing for wear.

Editing a Method:

· Press "SEP method file" and select the method you wish to edit. You can select the step you wish to edit by entering the number after the period then press do, or press "step forward" or "step backward" until the step you wish to edit appears on the display.

· Use the ">>" or "<<" keys to move to the field you wish to edit. Press "CE" to clear it, then enter the new value.

· If you alter a method, return it to its original state, or make a note of it for other users.