

BioRad Electroporator

Gene Pulser Xcell (PSC 543)

Micropulser (NSC 338)

Every time you use the electroporator, you MUST sign the log book.
Make sure everything (cuvettes, tubes, cells) is VERY COLD.
No salt in DNA.

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1. Keep cells cold on ice. You can either buy or make your own competent cells. Aliquot cells and store at -70°C . Good for ~6 months. Thaw only 1 time. Thaw on ice. Cuvettes and DNA on ice.
2. No salt in DNA mix.
3. The amount of DNA and competent cells depends on the cell type. See manual for details.
4. Turn Electroporator on (right side of Main unit).
5. Use #1 Exponential Protocol.
6. Enter.
 - a. Voltage – 2500 Enter
 - b. Capacitance – 25 Enter
 - c. Resistance – 400 or infinity Enter
 - d. Cuvette mm – depends on the electrode gap, mm
7. “P” flashing in lower right hand corner – ready to Pulse.
8. Put DNA and competent cells in the cuvette. Make sure there aren’t any bubbles. 100 ul total volume. Mix cells and DNA by pipetting up and down.
9. Open pod with tab. Put in cuvette. The cuvette has metal on 2 sides – line up metal sides with metal in pod. Close pod.
10. You should not reuse the cuvettes. If you do – no bleach and no autoclaving. Use EtOH to clean.
11. Preset Protocols. There are 9 pre-set bacterial protocols, 6 pre-set Fungal protocols and 12 pre-set mammalian protocols.
 - a. Using the keypad or the arrow keys, scroll through the list of names. For Bacterial and Mammalian protocols, use the right and left arrow keys to toggle between the 2 columns. When the protocol you want is highlighted, press Enter. Check out the Protocol Details to make sure the electroporation parameters are what you want. You can change the parameters for pulse and you don’t have to save the change (for a one time pulse).
12. Punch red button to pulse. The LCD display will blank and then say “Pulsing”. When the pulse is done, a tone will sound and the pulse measurements will be displayed on the Protocol Results screen
13. Check time constant. It should be ~10-15 msec. If too high – problem.
14. Use the Left and Right arrow keys to toggle between the Protocol Results screen and the last Protocol Detail screen.
15. After Pulse, place cells and DNA in recovery media (enriched media). Wash several times and spin several times 5 – 10 min each.
16. New program. Go to # 5 from the home screen or move cursor to User Protocols. You can also modify a pre-set protocol and save it under User protocols. For instructions for doing these 2 - please use manual.