Mini Prep Cell and Model 491 Prep Cell Starter Kit Instructions

This is your Prep Cell starter kit. The kit has been provided to demonstrate how well the Prep Cell resolves two adjacent proteins and familiarize you with the assembly and operation of the Prep Cell. The sample in the starter kit consists of two prestained proteins, Soybean Trypsin Inhibitor (apparent MW 27,500 +/- 10%) and Carbonic Anhydrase (apparent MW 32,500 +/- 10%). Migration of these two blue proteins will demonstrate how straight these bands will migrate during the run. This run can be monitored. It is important to note that as a consequence of the staining process, the bandwidths of the prestained proteins are greater than those of unstained proteins.

Gel composition: 10% T/2.67% C - Resolving gel
4% T/2.67% C - Stacking gel

Gel length:	**Mini Prep Cell**	**Model 491 Prep Cell**
Resolving gel:	5.5 cm (2 ml)	5.5 cm (20 ml)
Stacking gel:	0.5 cm (200 µl)	0.5 cm (2 ml)
Gel tube size:	7 mm ID	28 mm ID
Sample load:	30 µl	300 µl (~150 µg of each protein)

All reagents necessary for three trial runs are included in this kit. It is advisable to perform at least one trial run to familiarize yourself with assembly and operation of the Prep Cell. Detailed diagrams and instructions are provided in the Prep Cell instruction manual. Please read the manual before your first run.

Reagent Preparation, Assembly, and Operation

Step 1: **Prepare 30% T/2.67% C acrylamide monomer stock solution (100 ml)**
0.8 g bis
29.2 g acrylamide
Dissolve in 70 ml distilled water. Add distilled water to a final volume of 100 ml. Filter and store at 4 °C (30 days maximum).

Step 2: **Prepare 4x resolving gel buffer - 1.5M Tris/HCl, pH 8.8 (100 ml)**
18.17 g Tris base
Dissolve in ~ 80 ml distilled water.
Adjust to pH 8.8 with 10 N HCl (DO NOT back titrate with base). Add distilled water to a final volume of 100 ml. Store at room temperature.

Step 3: **Prepare 4x stacking gel buffer - 0.5M Tris/HCl, pH 6.8 (100 ml)**
6.0 g Tris base
Dissolve in ~80 ml distilled water.
Adjust to pH 6.8 with 10 N HCl. (DO NOT back titrate with base).
Add distilled water to a final volume of 100 ml.
Store at room temperature.

Step 4: **Prepare electrophoresis/elution buffer - Tris/Glycine/SDS (10 liters)** Dilute 1 liter of 10x Tris/Glycine/SDS buffer with 9 liters of distilled water.

Step 5: **Secure the gel tube assembly and cooling core to the casting stand and set up the cooling path needed during polymerization of the gel. See the instruction manual for details.**

Step 6: **Prepare resolving gel - 10% T/2.67% C, 0.375 M Tris/HCl, pH 8.8.**

30% T/2.67% C stock solution	6.67 ml
1.5 M Tris/HCl, pH 8.8	5.0 ml
Distilled water	8.27 ml
10 % ammonium persulfate	50 µl*
TEMED	5 µl*
TOTAL MONOMER	20 ml

* Degas monomer solution prior to adding catalyst.

Step 7: **Pour the degassed monomer solution with the added catalyst into the gel tube assembly.** Carefully overlay with water or water saturated 2-butanol or tert-amyl alcohol. After ~ 2 hours replace overlay with 0.375 M Tris/HCl, pH 8.8 buffer and continue polymerization overnight. See the instruction manual for details.

Step 8: **Prepare stacking gel - 4% T/2.67% C, 0.125 M Tris/HCl, pH 6.8**

30% T/2.67% C stock solution	1.33 ml
0.5 M Tris/HCl, pH 6.8	2.5 ml
Distilled water	6.1 ml
10 % ammonium persulfate	50 µl*
TEMED	10 µl*
TOTAL MONOMER	10 ml

* Degas monomer solution prior to adding catalyst.

Step 9: **Carefully decant or aspirate the overlay buffer and add the stacking gel monomer solution.** Carefully overlay with water, water saturated 2-butanol, or tert-amyl alcohol. Allow the gel to polymerize for 1–2 hours.

Step 10: **Carefully decant or aspirate overlay.** Assemble the elution chamber and the Prep Cell as described in the instruction manual.

Step 11: **Add electrophoresis buffer to the lower and upper buffer chambers and to the elution buffer reservoir.**

Step 12: **Apply sample.** Heat the sample to 40 °C for 1 minute to dissolve any solids which may have precipitated during storage. Using the sample application syringe, load the prestained proteins directly on the surface of the stacking gel, 300 µl for Model 491 Prep Cell and 30 µl for the Mini Prep Cell.

Recommended running conditions and expected results:

	Mini Prep Cell	**Model 491 Prep Cell**
Power conditions:	4–5 mA constant, or 1 W constant	40 mA constant or 10–12 W constant
Elution flow rate:	0.1 ml/min	1 ml/min
Cooling flow rate:	N/A	80-100 ml/min
Fraction collector:	0.25 ml/fraction	2.5 ml/fraction

- The ion front and the free dye should elute in 2–2.5 hours.
- Prestained Soybean Trypsin Inhibitor should elute after 2.5–3 hours, and prestained Carbonic Anhydrase after 3–3.5 hours.
- Total electrophoresis time is approximately 4 hours.

Product Information

Catalog Number	Description
161-5104	**Mini and Model 491 Prep Cell Starter Kit with Standard**
161-5101	**Mini and Model 491 Prep Cell Starter Kit without Standard**
161-0323	**Mini and Model 491 Prep Cell Standard**

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