SOP: SP010.2
Modified 3/9/18 by MCL

Performing Two-dimensional Electrophoresis Using the Life Technologies (Invitrogen) ZOOM IPG System (note 1)

Materials: (note 2)

1. 100-200µg of dry protein sample for IEF (note 3)
2. Deionized 8M Urea (note 4)
3. Sample Rehydration Buffer (note 5)
4. ZOOM Strips pH 4-7 (ZM0012: 12 Strips)
5. ZOOM Carrier Ampholytes pH 3-10 (10 ml: ZM0021)
6. ZOOM Carrier Ampholytes pH 4-7 (10 ml: ZM0022)
7. ZOOM IPGRunner Mini-Cell (ZM0001 Stored in C210)
8. UltraPure Urea (15505-035)
9. ZOOM CHAPS (ZC10003)
10. MilliQ Water
11. NuPAGE Sample reducing Solution 10x (NP0009)
12. ZOOM IPGRunner Cassettes, (ZM0003 10 units)
13. LDS sample equilibration buffer (4X: NP0008) (note 6)
14. Novex IEF Cathode (10X: LC5310) and Anode buffers (50X: LC5300)
15. NuPAGE MES SDS Running Buffer (20X: NP0002)
16. ZOOM Equilibration tray (ZM0007)
17. NuPAGE Novex 4-12% Bis-tris ZOOM Gel (NP0330BOX)
    or
18. Novex 4-20% Tris-Glycine ZOOM Gel (EC60261BOX)
19. XCell *SureLock* Mini-Cell (EI0001)
20. BIO-RAD Programmable Gel runner for Performing First and Second Dimension
21. EC Apparatus

Protocol

Step 1: Preparing samples for ZOOM IPG Strip Rehydration

1._____ Prepare the protein sample of interest at the correct concentration in a suitable volume of Sample Rehydration Buffer (notes 5 & 7).

2._____ Solubilize the protein sample at 4°C for at least 8 hours (note 8).

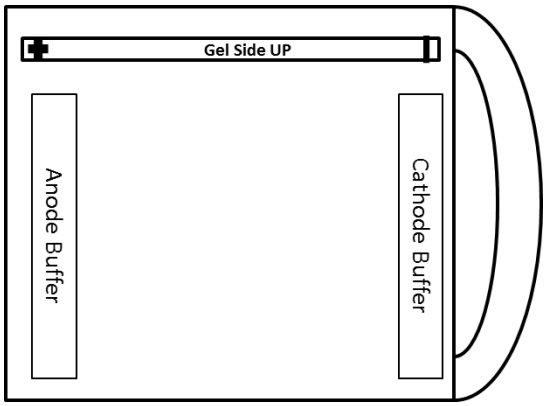
3._____ Centrifuge the solubilized protein sample at 14,000 rpm for 15 min at room temperature.

4._____ Set the ZOOM IPGRunner Cassette on a level surface.

5._____ Load sample rehydration buffer containing the protein sample into the sample loading wells located at the rounded edge of the ZOOM IPGRunner cassette (i.e. the cathode end).

6._____ Remove the ZOOM IPG Strip card from its pouch and by holding the ZOOM Strip at the basic end (-) overhanging plastic with a forceps peel the ZOOM IPG Strip away from the card backing

7._____ With the printed side facing *down* (i.e., gel side up) gently slide the acidic end (+) of the strip into the sample well at the curved end of the ZOOM IPGRunner Cassette (Cathode End) until the acidic end (+) of the strip touches the end of the channel slot. The diagram depicts the correct orientation of cassette and gel strip (note 9).



8._____ Repeat Steps 6 and 7 for the remaining wells containing sample (note 10).

9._____ Seal all Sample Loading Wells (including unused wells) with the Sealing Tape provided. Make sure that the tape fully seals all wells. Smooth the tape down to create a sealed environment for rehydration.

10._____ Rehydrate the ZOOM Strips in the presence of the proteins sample for 8-16 hours at room temperature.

Step 2: Performing Isoelectric Focusing

11._____ Remove the Sealing Tape and Sample Loading Devices from each end of the cassette to expose the adhesive backing.

12._____ Using the black alignment marks as a guide, place an Electrode Wick at each end of the ZOOM IPGRunner Cassette.

13._____ Apply 1 ml of a diluted Cathode buffer to the top wick, and 1 ml of diluted Anode buffer to the bottom wick. Blot off the excess with a KimWipe (note 11).

14._____ Slide the cassette into position against the ZOOM IPGRunner Core such that the electrode wicks are in contact with the electrodes of the core.

15._____ Repeat Step 14 with a second ZOOM IPGRunner Cassette (if applicable) or use the Buffer Dam in place of a second ZOOM IPGRunner Cassette.

16._____ Slide the sandwich containing the ZOOM IPGRunner Core/ZOOM IPGRunner Cassettes (or Buffer Dam) into the Mini-Cell Chamber of the ZOOM IPGRunner.

17._____ Insert the Gel Tension Wedge into the Mini-Cell behind the ZOOM IPGRunner Core.

18._____ Pull the Gel Tension lever of the wedge toward the front of the ZOOM IPGRunner Mini-Cell until the lever comes to a firm stop.

19._____ Fill the Outer Chamber of the Mini-Cell with 600 ml of MilliQ water (note 12).

20._____ Place the ZOOM IPGRunner Cell Lid on the ZOOM IPGRunner Core.

21._____ Connect the electrode cords to the power supply, making sure to plug the red electrode into the red jack and the black electrode into the black jack.

22._____ Plug an EC Apparatus into another set of jacks in the power supply in order to provide necessary resistance.

23._____ Perform Isoelectric Focusing (note 13).

Step 3: Performing Second Dimension (note 14)

24._____ Remove the protective plastic cover of the IPG cassette and replace it with a ZOOM equilibration tray (note 15).

25._____ Incubate the IPG strip(s) in Reducing Solution for 15 to 30 minutes at room temperature, with gentle rocking (note 16).

26._____ Decant the Reducing Solution using the spouts on the tray. Discard the solution.

27._____ Where appropriate for the particular protein sample of interest an additional Alkylation step should be preformed prior to proceeding with the second dimension (note 17).

28._____ Assemble one ZOOM NuPAGE Novex IPG-well gel (note 18) per ZOOM Strip into the XCell *SureLock* Mini-Cell (note 19).

29._____ Fill the Upper and Lower Buffer Chambers with the appropriate 1 X Running buffer (note 20).

30._____ Remove each ZOOM Strip from the cassette using a forceps by holding the plastic overhang at the Cathode end.

31._____ Carefully remove the Anode end plastic overhang flush with the gel using sharp scissors.

32._____ Position the ZOOM IPG strip into the well such that you can read the writing (plastic side facing front, + on the left, - on the right).

33._____ With the Anode end of the strip in place, carefully rest the plastic backing of the gel strip against the plastic of the gel cassette so that you can remove the Cathode end plastic overhang using sharp scissors.

34._____ Align the ZOOM IPG strip properly in the ZOOM Gel well using a thin spatula or forceps. Avoid introducing any air bubbles (note 21).

35._____ Load an appropriate volume of molecular weight standards of choice in the marker well.

36._____ Place the XCell *SureLock*™ Mini-Cell lid on the Buffer Core, connect the electrode cords to the power supply, noting polarity.

37._____ Perform the Second Dimension at a constant voltage (note 22).

38._____ At the end of electrophoresis, turn off the power and disassemble the XCell *SureLock* Mini-Cell.

39._____ Rinse and cool the gel in running DI-Water

40._____ Remove the Gel from the pre-cast plastic holder and carefully remove the protruding gel piece located at the bottom of the gel.

41._____ Stain the Gel with a method of choice (SimplyBlue SafeStain (see product bottle for instructions), or silver staining (SOP: SP012) or perform western transfer and immunoblotting (SOP: SP011).

Notes:

1. The buffer formulations, reagents described and instructions contained within this SOP have been successfully applied to a number of different protein samples in the Dobos Lab using predominantly the 7cm ZOOM Strips pH 4-7 with appropriate ZOOM Carrier Ampholytes. As such the protocol provides a robust and well tested initial starting point. However, it must be noted that further optimization may be required to successfully perform IEF on the particular protein fractions of interest. Such optimization should be performed following consultation initially with lab members familiar with the techniques or more pertinently with senior lab personnel who participated in the development of the protocol. Similarly, interested individuals should seek guidance from lab members experienced in the protocol while performing the protocol for the first time.

2. All reagents used must be of the highest quality available and should be routinely replaced either when the expiration date has been reached or for “in house” prepared solutions every 3 months.
3. As a guide: for downstream silver staining of gels use 100µg, for Commassie or SimplyBlue staining use 200 µg of protein sample.
4. Urea should be deionized prior to use to remove reactive cyanate ions. 8 M solutions of urea may be bulk deionized using an ion exchange resin. Add AG-501-X8 (Bio-Rad, Hercules, CA) at 5 g/100 mL, stir the solution for 2 h at RT, and filter the solution to recover deionized 8 M urea.
5. Sample Rehydration Buffer (for pH 4-7 ZOOM Strips):

Reagent (Stock Conc.)	**Final Concentration**
CHAPS (10% prepared in 8M Urea)	1%
NuPAGE Sample Reducing Agent (10X)	20mM DTT
ZOOM Ampholytes pH 4-7	0.75%
ZOOM Ampholytes pH 3-10	0.25%
8M Urea	Final Volume diluent

6. LDS sample equilibration buffer will crystalize after storage at 4°C, and will resolublize when brought to room temperature. Five ml aliquots should be made in order to speed the process.
7. The final volume of sample rehydration solution containing a particular protein sample should not exceed 200 µl, with a minimum of 160 µl required for adequate strip rehydration.
8. Over the 8 hour solubilisation incubation, intermittent bath sonication of the protein sample for 5 min at room temperature enhances the solubilisation of the protein sample.
9. Avoid introducing large air bubbles while sliding the ZOOM Strip into a sample well. To remove large bubbles, slide the strip gently back past the bubble, then slide it forward slowly pushing the air bubble to the end. Small air bubbles do not adversely affect rehydration.
10. If you are not using all sample wells there is no need to fill the remaining wells with sample buffer, unused wells should be left empty.
11. A 1/100 dilution of the Stock Cathode and Anode buffer is used, prepared in MilliQ water. Ensure that the wells on each end of the cassette are completely covered by the electrode wicks before progressing to the addition of the Cathode and Anode buffer.
12. Do not pour any other liquid into the Inner Chamber of the Mini-Cell, and ensure that there is no leakage of water into the inner core before proceeding.
13. The Standard 1st Dimension isoelectric focusing use the following parameters, please note that alterations to this program set may be required for optimal Isoelectric focusing (it may be necessary to extend step 4 to 30 minutes to prevent power failure at 2000V):
    1) 250V 4mA 4W 10 min,
    2) 450V 3mA 4W 10 min,
    3) 750V 2mA 4W 10 min,
    4) 1000V 1mA 4W 10 min,
    5) 2000V 1mA 4W 2 hrs.
14. When the IEF of protein samples has been completed proceed directly with ZOOM strip equilibration in Reducing (and where appropriate Alkylating) buffers prior to performing the second dimension SDS/PAGE using ZOOM Gels.
15. Remove the plastic covering by firmly griping it at the cathode (+) end of the cassette and carefully peeling it away. Place the equilibration tray over the wells, and carefully seal around the edges to prevent leakage of the equilibration buffer(s). Alternatively the strips can be removed from the cassette and equilibrated in a buffer reservoir.
16. To prepare Reducing Solution, add 500 µl NuPAGE® Sample Reducing Agent (10X) to 4.5 ml of the 4X NuPAGE® LDS Sample Buffer.
17. Add 5 ml Alkylating Solution (116 mg Iodoacetic acid in 5 ml 4X NuPAGE LDS sample buffer) to the IPG strip. Incubate for 15-30 minutes at room temperature and remove the Alkylating solution.
18. The choice of gel is dependent on the molecular weight range of the protein sample of interest, with the Novex 4-12% Bis-Tris Gel used to resolve the broadest range of proteins. Novex 4-20% Tris-Glycine ZOOM Gels have also been used.

19. The XCell *Surelock* Mini-cell can accommodate two ZOOM IPG well Gels or one ZOOM Gel and a buffer dam.
20. The choice of running buffer is dependent on the primary choice of ZOOM Gel; in general Invitrogen MES buffer (1X) has been used in conjunction with the ZOOM 4-12% Bis-TRIS Gel with the Invitrogen MDS (1X) used in conjunction with the ZOOM 4-20% Tris-Glycine gel buffer.
21. While not routinely used, the preparation of an Agaraose sealing solution followed by sealing the strip into the well can be preformed prior to running the second dimension.
22. For NuPAGE Novex Bis-Tris ZOOM Gel second dimension is preformed at 200 constant volts for approx. 35-40 minutes or for NuPAGE Novex Tris-Glycine ZOOM Gel at 125 constant volts for 90 minutes for. In both cases the sample should be run until the dye fronts reach the protruding gel piece at the end of the gel.