1.0 Overview

1.1 This SOP is written to detail the steps taken to prepare a tissue sample for analysis by MS using ZOOM isoelectric focusing (IEF).

2.0 Reagents and Materials

2.1	Urea	Fisher Scientific - Cat#BP169-500
2.2	Acetonitrile (ACN), HPLC Grade	Fisher Scientific - Cat#A998-1.
2.3	Water, HPLC Grade, J.T. Baker	VWR – Cat#JT9823-1

3.0 Apparatus/Instrumentation

3.1	ZOOM IPGRunner Mini-Cell	Invitrogen - Cat# ZM0001
3.2	ZOOM IPGRunner Cassettes	Invitrogen - Cat# ZM0003
3.3	ZOOM Custom Order pH Strips (pH 3.5-4.7)	
3.4	Invitrogen ZOOM Dual Power (100-120 VAC 47 – 60 Hz) œ	Invitrogen - Cat# ZM10001
3.5	Nunc deep well plates, 2mL, sterile	Nalge Nunc International - Cat#278743
3.6	Falcon 96 well Microplates, clear, flat-bottom with lid, sterile, polystyrene	BD Biosciences - Cat#351172

4.0 Procedure

4.01 Dependant on how much protein you want to run on a single Zoom strip, you should either: Reconstitute your sample(s) in water and aliquot what you need to run, and then dry that down in the speed vac and then reconstitute in 155uL 6M Urea

(or)

Take the dried sample from 4.16 and directly reconstitute dry samples in 155uL 6M Urea. Vortex samples vigorously for one minute. Centrifuge as necessary.

(Note: If you are digesting large amounts of protein and only need aliquots for the next steps, DO NOT store samples in UREA. You should store the samples in water.)

(Note: Make Urea fresh daily.)

6M Urea = molecular weight (60.06g) x 6 = 360.36g/ 1L

6M Urea = 0.72g/2mL

4.02 Load sample (155uL) into the sample loading wells located at the rounded edge of the ZOOM IPGRunner cassette. (You do not need to load the remaining wells if you are not planning on using them for rehydration…..leave the unused wells empty.)

4.03 Remove Zoom strip (pH 3.5-4.7) from card holding from the basic (-) end, flipping and making the strip gel side up.

4.04 Using your fingers, gently slide the acidic (+) end of the strip into the sample well located on the curved side of the cassette. (To avoid air bubbles, slide the strip back and forth until all bubbles are gone.)

4.05 Seal all sample wells with the purple sealing tape provided in the kit.

4.06 Incubate at room temperature for at least 1 hour.

4.07 Remove sealing tape and white sample loading device from cassette to expose adhesive.

4.08 Apply (600uL) DI water to 2 electrode wicks each.

4.09 Place an electrode wick at both ends of the cassette over the adhesive, using the black alignment marks.

4.10 Slide the cassette into position into the ZOOM IPGRunner core rounded side up, and the wicks will be in contact with the electrodes of the core. (If only 1 cassette is being focused, use the buffer dam in the core, however, if 2 cassettes are being focused, the buffer dam is not needed)

4.11 Slide the apparatus with the core, cassette, and buffer dam into the mini-cell chamber core.

4.12 Pull the gel tension lever of the wedge toward the front of the mini cell until the lever tight.

4.13 Fill the **OUTER** chamber of the mini cell with 600mL DI H2O. **CAUTION: Do not pour any other liquid into the inner chamber of the mini cell.**

4.14 Place the ZOOM IPGRunner cell lid on the ZOOM core.

CAUTION: Do not handle the lid with the electrode cords plugged into the power supply.

4.15 With the power supply turned off, connect the electrode cords to the power supply.
Turn the power on and perform IEF with the following method: (it is preloaded on the power supply under method #1) 175V for 15 min
175-2000V ramp for 45 min.
2000V for 105 min.

Note: (Make sure the power supply is working before you leave the strips to focus. When the method starts, look to see if there are mAMPS, if not the strips are not focusing and the power supply needs troubleshooting.)

4.16 The run is 2 hours and 45 min. total. The power will automatically cut off. Turn the power off and disconnect the cables from the power supply. Remove the lid and pour off the water. Unlock the gel tension wedge and remove the ZOOM cassette.

4.17 Carefully peel off the clear film to get to the strips.

4.18 Cut each strip into 10 fractions and place gel side up in a 96 well plate.
(Cut strips can be stored in the -80° freezer.)
