SOP: SP007a
Modified 9-12-19 by MCL

Running SDS-PAGE Gels
Using Invitrogen Mini Gel Tank

Materials and Reagents:

1. 2-5 µg protein sample
2. 20X MES Running Buffer
3. Molecular weight marker (note 1)
4. 5X Sample Buffer (note 2)
5. 4-12% Invitrogen/Novex Bis Tris SDS-PAGE gel (1.0mm, 15well)
6. 0.65 ml Eppendorf Tubes
7. 100°C Heating Block
8. Microcentrifuge
9. Invitrogen/Novex Gel knife
10. All items in Table 1

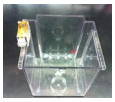
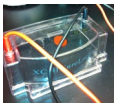
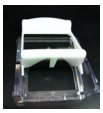
Power Supply with adapter	Invitrogen Novex Mini-cell	X-cell Surelock lid	Buffer Dam	Gel loading tip	Buffer Core	Gel Tension Wedge
						

Table 1 – Supplies needed for Polyacrylamide Gel Electrophoresis

Protocol:

1._____ Prepare protein samples to run on gel by aliquoting 2-5 µg of protein (note 3) into a 0.65 ml eppendorf tube.

2._____ Add 2 µl of 5X sample buffer to each sample.

3._____ Fill to 10 µl with appropriate buffer (usually 10mM ambic, water, or PBS) (note 4).

4._____ Boil samples on the 100°C heating block for 5 minutes while gel chamber is being prepared.

5._____ Prepare 400 ml of MES running buffer for each gel by taking 20 ml of the 20X MES buffer and qs to 400 ml with MilliQ H_2O (note 5).

6._____ Prepare gel by taking 4-12% Bis Tris SDS-PAGE 15 well 1.0 mm gel out of storage pouch. Gently remove comb and remove tape from gel. Rinse out the wells of the gel with di-H_2O or with 1X MES buffer. If the well lanes are not straight, they can be adjusted using a gel loading tip.

7._____ Assemble Mini Gel Tank as shown in Figure 1 (note 6).

8._____ Place the gel into the chamber with the wells are facing forward (note 7) and, with the gel slightly raised, clamp it into place.

9._____ Fill all wells with 1X MES buffer.

10._____ Briefly centrifuge the boiled samples in a microcentrifuge to collect all of the sample in the bottom of the tube.

11._____ Load molecular weight marker and protein samples into the gel wells using a gel loading tip.

12._____ Release the clamp, lower the gel to the bottom of the tank, and close the clamp.

13._____ Gently pour the prepared 1X MES buffer to the fill line in the tank.

14._____ Make sure the power supply is turned off and your gloves are dry. Place the lid on the Mini Gel Tank. Plug the electrode cords from the lid into the power supply, matching **red**-to-**red** and **black**-to-**black** (note 8).

15._____ Turn on the power supply.

16._____ Program the power supply parameters as follows (note 9):

Volts:	200V (constant)
Time:	30-35 min
Current:	mA are variable (not programmed)

17._____ Push the RUN button. Bubbles should emit from the cathode core.

18._____ The gel is finished running when the dye-front has reached the foot of the gel.

19._____ Turn off power supply and disconnect electrodes from power supply. Empty the MES running buffer into the sink and wash the entire unit including gel in di-H_2O.

20._____ Use the gel knife to gently crack open the two plastic plates of the gel cassette. Use the gel knife to cut off the foot and wells of the gel and discard these into a hazardous waste container.

21._____ Gels can be transferred by western blot (SOP: SP011), stained by SimplyBlue SafeStain (see product bottle for instructions), or stained by silver staining (SOP: SP012).

Notes:

1. If the gel is to be transferred for western blot (SOP SP011) use 5 µl prestained markers (Biorad, cat# 161-0374). If staining the gels, no more than 3 µl of prestained marker should be used (alternatively, use 5 µl unstained markers (Biorad, cat# 161-0363)).
2. <u>5X Sample Buffer</u>
   0.36 g Tris-Base
   5.0 ml Glycerol
   1.0 g SDS
   2.5 ml β-Mercaptoethanol
   5 mg Bromophenol Blue
   QS to 10 ml with Water
   Rock overnight at room temperature. Make 1ml aliquots and freeze until ready to use.
3. Approximately 2 µg of pure protein, or 5 µg of crude material (i.e. subcellular fractions) should be run on a gel. If your sample is at a very low concentration, it may be necessary to dry the sample on the savant (see SOP SP005 for operation of the Savant) and resuspend in 8 µl water.
4. The maximum volume that a 15-well 1.0 mm Novex Bis Tris gel will hold is 15µl per well.
5. Make each 400 ml running buffer separately to ensure that all gels receive an equal amount of buffer salts.
6. The cassette clamps should be installed with the anode connector facing the center of the tank. If only running one gel, remove the other cassette clamp.
7. This configuration is different from the Novex Mini-Cell, so be sure that you are familiar with which unit you are using. When the wells are facing you, the text on the gel cassette will be facing away from you.
8. Ensure that the power supply port that you are using has adapters installed that fit the Mini Gel Tank electrodes.
9. Run conditions listed are for 4-12% Bis-Tris gels in MES buffer. Other types of gels and/or running buffer may require different run conditions. Consult product information.

Troubleshooting Guide:

Problem	Possible Cause	Remedy
Sample does not migrate through gel *and/or* No bubbles form in the buffer core *and/or* "E1" error message on power supply	Tape left on gel	Remove tape and set gel back up. If this is done very carefully without disturbing the samples, the gel can still be run.
	Buffer level too low (not making contact with the top of the gel)	If necessary, reposition the gel/dam to correct significant leaks. Fill buffer core completely, being careful not to disturb the samples in the wells. Restart run.
Dye-front "frown"	Poorly mixed running buffer	This gel is not recoverable. For repeat run, thoroughly mix running buffer before use. If running more than one chamber, make each 400 ml running buffer separately
	Buffer level too low (making uneven contact with the top of the gel)	If the problem is minor, fill buffer core completely and restart run. If the "frown" is significant, the gel may not be recoverable.
Sample disappears after the start of the run	Electrodes plugged in backward	This gel is not recoverable. For repeat run, check that the red electrode is plugged into the red adapter AND that the red adapter is plugged into the red jack.
Bubbles develop in the gel during run	Gel was previously frozen	Gel may still run, but use caution during downstream steps, as the gel will be extremely fragile. Check remaining gels in the box for signs of freezing

References:

http://tools.invitrogen.com/content/sfs/manuals/nupage_minigel_qrc.pdf

http://www.invitrogen.com/etc/medialib/en/filelibrary/protein_expression/pdfs.Par.92119.File.dat/CO31134-NuPAGE_vs_TGX_AppNt.pdf

http://tools.invitrogen.com/content/sfs/manuals/electrophoresisguide_man.pdf

http://tools.invitrogen.com/content/sfs/manuals/surelock_man.pdf

Nature. 1970 Aug 15;227(5259):680-5. *Cleavage of structural proteins during the assembly of the head of bacteriophage T4*. Laemmli UK.