SOP: SP007.3
Modified 6.15.2023 by KCB

Running SDS-PAGE Gels

Materials and Reagents:
2-5 µg protein sample
20X MES Running Buffer (Fisher Scientific #NP0002)
Molecular weight marker (note 1)
4X Lithium Dodecyl Sulfate (LDS) Sample Buffer (Fisher Scientific #NP0008, note 2)
4-12% Invitrogen/Novex Bis Tris SDS-PAGE gel (1.0mm, 15well, Fisher Scientific #NP0323BOX)
0.65-1.5 mL Eppendorf Tubes
70°C Heating Block
Microcentrifuge
Gel knife
All items in Table 1

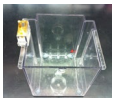
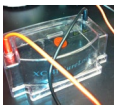
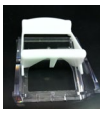
Power Supply with adapter	Invitrogen Novex Mini-cell	X-cell Surelock lid	Buffer Dam	Gel loading tip	Buffer Core	Gel Tension Wedge
						

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 an 0.65-1.5 mL Eppendorf tube.

2._____ Add 2.5 µl of 4X sample buffer to each sample.

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

4._____ Heat samples in the 70°C heating block for 10 minutes while gel chamber is being prepared.

5._____ Prepare 500 ml of MES running buffer by taking the 25 ml of the 20X MES buffer and qs to 500 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 the white tape from the bottom of the gel. Rinse out the wells of the gel with di-H_2O. If the well lanes are not straight, they can be adjusted using a gel loading tip.

7._____ Assemble Novex mini cell as shown in Figure 1 and 2. The upper chamber is formed by the buffer core and the two gels (or one gel and buffer dam).

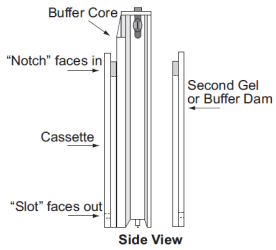


Figure 1: Upper chamber assembly

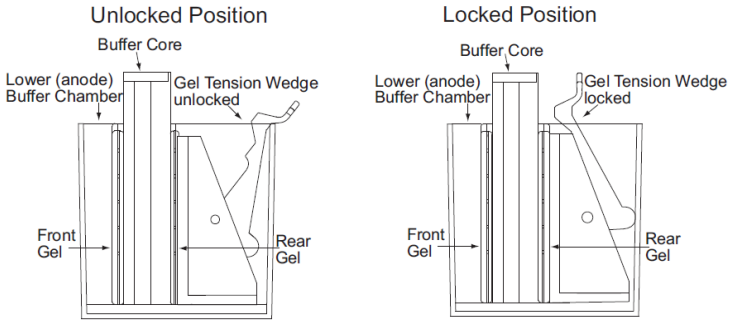


Figure 2: Upper chamber locked into outer chamber

8._____ Pour the prepared 1X MES buffer into the upper chamber until full and then fill the lower buffer chamber with the remaining buffer by letting excess buffer from the upper chamber gently flow into the lower chamber. The lower chamber does not need to be completely full as long as there are no leaks in the system and the buffer level in the upper chamber does not fall. Make sure that the wells in the gel are completely covered in buffer.

9._____ Briefly centrifuge the heated samples in a microcentrifuge to collect all of the sample is in the bottom of the tube.

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

11._____ Make sure the power supply is turned off and your gloves are dry. Place the Novex mini-cell lid on the chamber. Plug an adapter into the power supply, then plug the electrode cords from the mini-cell lid into the adapter, matching **red**-to-**red** and **black**-to-**black**.

12._____ Turn on the power supply.

13._____ Program the power supply parameters as follows (note 5):

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

14._____ Push the RUN button. Bubbles should emit from the buffer core wire.

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

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

17._____ 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.

18._____ 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 2 µl of prestained marker should be used (alternatively, use 5 µl unstained markers (Biorad, cat# 161-0363)).
2. Before storing the sample buffer, 200 µl of 2-β-Mercaptoethanol (BME) is added to 800 µl of 4x LDS sample buffer. 1 ml of 4X LDS Sample buffer + BME is aliquoted into 1.5 ml Eppendorf tubes for storage

at 4°C.

3. Approximately 2 µg of pure protein, or 5 µg of crude material (i.e. subcellular fractions or culture filtrate proteins) 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. If running more than one chamber of gels, make each 500 ml running buffer separately to ensure that all chambers receive an equal amount of buffer salts.
6. 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 500 ml running buffer separately
	Sample too acidic/basic/salty	Perform a buffer exchange with a small spin column into a more neutral and less salty buffer, like ambic, PBS, or water with 1X LDS
	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

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