SOP: SDS-PAGE Protein Gel Electrophoresis

Approvals:

Preparer: Robin M. Zuck Date: 15APR19
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Reviewer: Dr .Maggie Bryans Date: 20APR19

1. **Purpose:**
   1.1. To describe the steps necessary to perform an SDS-PAGE analysis of a protein sample

2. **Scope:** This SOP covers the preparation of SDS-PAGE protein gels suitable for Coomassie staining or Western Blotting.
3. **Responsibilities:**
   3.1. It is the responsibility of the course instructor/lab assistant to ensure that this SOP is performed as described and to update the procedure when necessary.
   3.2. It is the responsibility of the students/technician to follow the SOP as described and to inform the instructor about any deviations or problems that may occur while performing the procedure.
4. **References:**
   4.1. XCell SureLock® Mini-Cell User Guide, Publication Part number IM-9003
   4.2. GelCode Blue Stain Reagent Instructions, Thermo Scientific
5. **Precautions:**
   5.1. Routine care should be exercised in the handling of buffers and samples of biological materials, which may have harmful biological activity in the case of accidental ingestion, needle stick etc.
   5.2. Gloves, a lab coat and protective eyewear should be worn when handling buffers and samples.
   5.3. Always wear gloves when handling polyacrylamide gels.
6. **Materials:**
   6.1. 4-20% Tris-Glycine Gel, Invitrogen Novex WedgeWell (Reference # XP04200BOX)
   6.2. 10X Tris/Glycine/SDS Running Buffer, BIO-RAD Catalog # 161-0732
   6.3. GelCode Blue Stain Reagent, Thermo Scientific (Product # 24590)
   6.4. XCell SureLock Gel Box
   6.5. Gel Knife
   6.6. Power Supply
   6.7. Heating block set at 95ºC
   6.8. 1.5 ml microfuge tubes
   6.9. 2X Laemmli Sample buffer, BIO-RAD catalog #161-0737 with added ß mercaptoethanol
   6.10. Precision Plus Protein Kaleidoscope Ladder, (BioRad catalog # 161-0375)
   6.11. Gel loading pipette tips
   6.12. 10 ml syringe and 21G2 needle
   6.13. Ice bucket and ice
7. **Procedure:**
   7.1. Prepare Samples.
   Note: protein samples should be kept on ice while preparing the samples.
      7.1.1. Fill the needed number of holes in the heating block with Milli Q water.
      7.1.2. Turn the heat block on and set the temperature to 95°C to preheat.

SOP: SDS-PAGE Protein Gel Electrophoresis

7.1.3. Label one microfuge tube for each sample.
7.1.4. For each sample to be analyzed;
7.1.4.1. Determine the amount of total protein to be loaded on the gel.
7.1.4.2. Using the sample total protein concentration, calculate the sample volume equal to the amount of protein to be loaded.
7.1.4.3. Calculate the volume of Milli Q water needed to add to the sample volume to bring the combined water + sample volume to 15µl.
7.1.4.4. Add 15 µl of 2X to this sample tube.
7.1.4.5. If the sample protein concentration is too low to add the desired protein amount in 15µl, it is possible to load 40µl in a well. Prepare the sample using a sample volume of up to 20 µl. If the sample volume needed is less than 20µl add enough Milli Q water to the tube to bring the volume to 20µl. Then add 20µl of 2X sample buffer to this tube. For these samples 40µl will be loaded into the well of the gel.
7.1.4.6. Combine the calculated sample, water and 2X sample buffer volumes for each sample in the labeled sample microfuge tube.
7.1.5. Heat each of the prepared sample tubes at 95°C in the heating block for 2 minutes.
7.1.6. Return the sample tubes to ice until they are loaded on the gel.
7.1.7. Just prior to loading give the sample tubes a quick spin in a table top centrifuge to collect the sample in the bottom of the tube.

7.2. Prepare 800ml of 1X Tris/Glycine/SDS Running Buffer.
7.2.1. Add 80 ml of 10X Tris/Glycine/SDS Running Buffer to 720 ml of MilliQ water
7.2.2. Mix gently to avoid foaming.
7.3. Prepare the gel/gels and assemble the gel box. See Attachment 8.1.
7.3.1. Cut open the gel cassette pouch and remove the gel.
7.3.2. Discard the gel packaging buffer.
7.3.3. Rinse the gel cassette with Milli Q water.
7.3.4. Remove the tape covering the slot on the back of the gel cassette.
7.3.5. Carefully to avoid damaging the wells, remove the comb from the top of the gel cassette by sliding the comb straight out.
7.3.6. Place the Buffer Core into the Lower Buffer Chamber
7.3.7. Place the Gel Tension Wedge into the Gel Box behind the Buffer Core. Make sure the Gel Tension Wedge is in the unlocked position. The Gel Tension Wedge should rest on the bottom of the lower buffer chamber. (See attachment 8.2)
7.3.8. Insert the gel cassette into the lower buffer chamber in front of the core with the shorter well side of the cassette facing the buffer core.
7.3.9. If you are running two gels place the second gel cassette in the lower buffer chamber behind the buffer core with the shorter well side of the cassette against the buffer core.
7.3.10. If you are running only one gel insert a Buffer Dam in place of the second, rear, gel cassette.
7.3.11. Pull the Gel Tension Wedge Lever toward the front of the Gel Box until it comes to a firm stop. The gels or gel and buffer dam should now be held firmly against the buffer core.

SOP: SDS-PAGE Protein Gel Electrophoresis

7.3.12. Fill the upper buffer chamber with 200ml of the 1X running buffer, use enough buffer to completely cover the sample wells.
7.3.13. Make sure that the upper buffer chamber is not leaking. If the buffer level drops, reseat the gels by repeating steps 7.3.8 through 7.3.11.
7.3.14. Fill the lower chamber with the remaining 600 ml of running buffer.
7.3.15. Using the syringe and 21G2 needle carefully flush each of gel wells with 1X running buffer.
7.3.16. Using gel loading pipette tips, carefully load the sample into the bottom of the designated well being careful not to introduce bubbles in the well.
7.3.16.1. Remember to load one well with 5µl of the Protein Ladder
7.3.16.2. Load each sample well with the with the total prepared sample volume, (either 30µl or 40µl).
7.3.16.3. Load any unused wells with 30µl of 1X sample buffer, (15µl of Milli Q water + 15µl 2X sample buffer).
7.3.17. Place the lid on the gel box and check that it is firmly seated.

7.4. With the power supply OFF, connect the electrodes from the gel box to the power supply as follows;
7.4.1. Connect the positive, red jack to the red port.
7.4.2. Connect the negative, black jack to the black port.
7.4.3. Turn the power on,
7.4.4. Set the power supply to constant voltage
7.4.5. Using the up and down arrows adjust the voltage to 125 volts.
7.4.6. The gel should run for 60 to 90 minutes. Monitor the progress of the dye front and turn the power supply OFF when the dye front is slightly above the gel foot. The colored bands of the ladder should be visible and separated.
7.4.7. With the power OFF, disconnect the power supply from the gel box.
7.4.8. Remove the lid from the box. Unlock the Gel Tension Wedge and remove the gel cassette.

7.5. Remove the gel.
7.5.1. Prepare a container for washing and staining the gel. It should be large enough to hold the gel with a volume of about 50ml. Place about 20 ml of Milli Q water in this container
7.5.2. Lay the gel cassette on the bench well side up. Insert the gel knife at the bottom corner of the cassette between the 2 plastic plates being careful not to contact the gel. Angle the knife up and down to separate the plates. You will hear a cracking sound. Place the knife in the opposite bottom corner and separate the plates on that side.
7.5.3. Carefully open the cassette, the gel will be attached to one of the plates. Discard the plate that is not holding the gel.

IF THE GEL IS TO BE USED FOR A WESTERN BLOT CONTINUE USING THE SOP FOR THE APPROPRIATE WESTERN BLOTTING PROCEDURE.

SOP: SDS-PAGE Protein Gel Electrophoresis

7.5.4. If the gel is attached to the shorter plate use the gel knife use the knife to gently lift a bottom corner of the gel. Using both hands to lift the gel transfer the gel to the prepared container with Milli Q.

7.5.5. If the gel is attached to the longer slotted plate, carefully use the knife to push the gel foot up through the slot so that the gel can be removed from the plate. Using both hands gently lift the gel and transfer the gel to the prepared container containing Milli Q water.

7.5.6. Allow the gel to wash in the for 5 minutes on a rotary platform. Replace the Milli Q water with 20 ml of fresh Milli Q and repeat this 5 minute wash twice more for a total of three washes.

7.6. If a Western Blot is to be prepared using this SDS-PAGE gel refer to SOP: XX for preparing a Western Blot.

7.7. Stain the gel using Coomassie Blue stain

7.7.1. Drain the water from the container holding the gel and add approximately 20 ml of GelCode Blue Stain. Cover the box with parafilm and place on a rotary plate. Incubate the gel with the stain for 6 hours to overnight.

7.8. Destain the gel

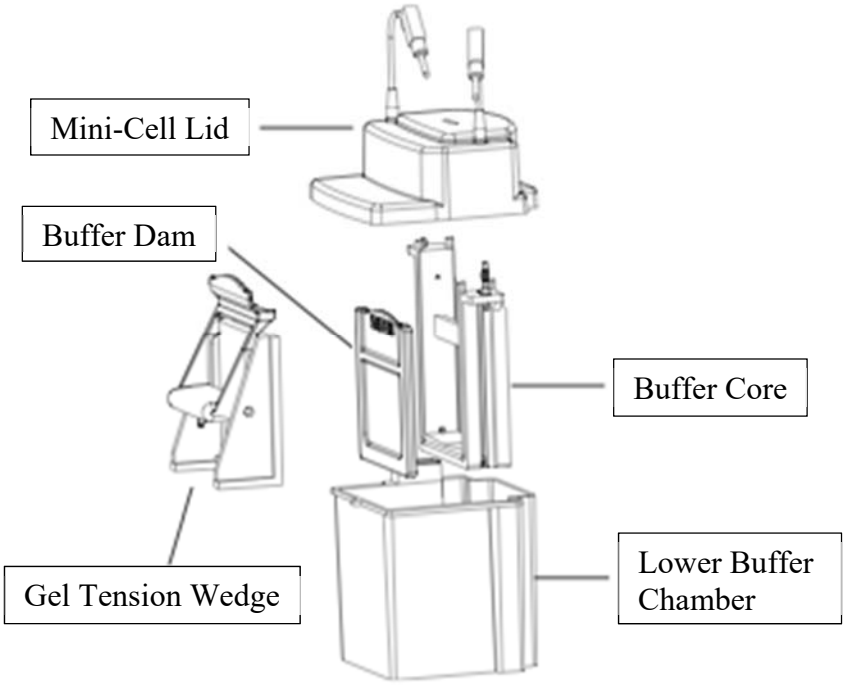
7.8.1. Decant the stain from the gel and add approximately 20 ml of Milli Q water and return to the rotary plate for 20 minutes. Repeat this wash process two more times for a total of 3 washes.

7.9. The dyed gel can now be imaged and analyzed.

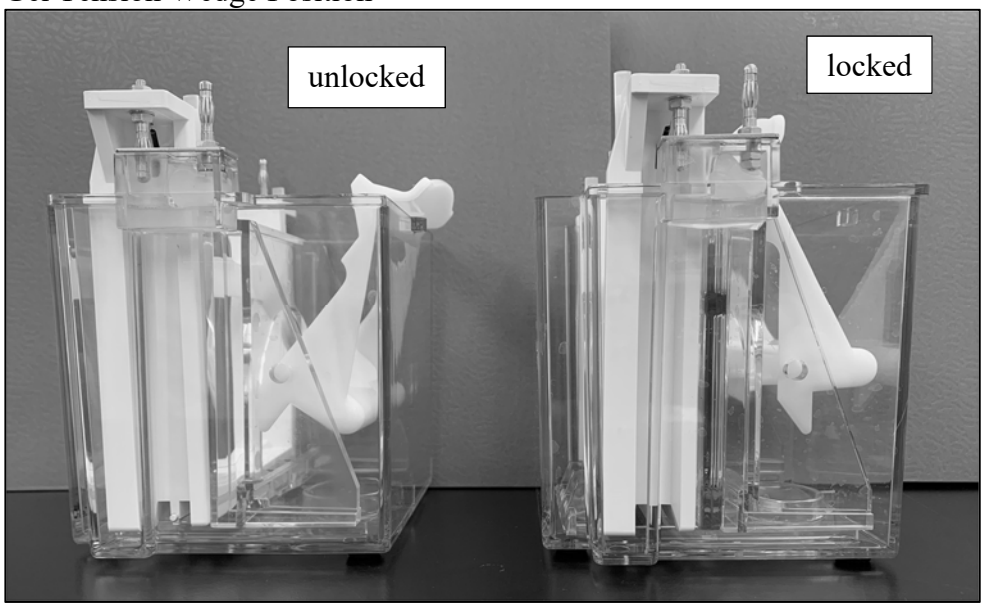
SOP: SDS-PAGE Protein Gel Electrophoresis

8. Attachments

8.1. Gel Box



8.2. Gel Tension Wedge Position



SOP: SDS-PAGE Protein Gel Electrophoresis

9. History:

Revision Number	Effective date	Preparer	Description of Change
0	20/04/19	Robin Zuck	Initial release