SOP: Analysis of rAAV purity by SDS-PAGE and Silver Stain

Approvals:

Reviewer: Hetal Doshi Date: 22MAY23
Reviewer: Dr. Maggie Bryans Date: 18JULY23

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

2. **Scope:** This SOP covers the preparation of SDS-PAGE protein gels suitable for silver staining
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. Pierce™ Silver Stain Kit Reagent Instructions, Thermo Scientific Catalog# 24612
   https://www.thermofisher.com/document-connect/document-connect.html?url=https://assets.thermofisher.com/TFS-Assets%2FLSG%2Fmanuals%2FMAN0016358_2161478_PierceSilverStainKit_UG.pdf
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. Pierce™ Silver Stain Kit Reagent Instructions, Thermo Scientific Catalog# 24612
   6.4. XCell SureLock Gel Box
   6.5. 50ml Fixing solution (30ml ultrapure water,+ 15ml 95% Ethanol, + 5ml acetic acid)
   6.6. 50ml 10% Ethanol
   6.7. 50ml 5% Acetic acid as a stop solution
   6.8. 50ml conical tube quantity 3
   6.9. Ultra-pure water
   6.10. Gel Knife
   6.11. Power Supply
   6.12. Heating block set at 95ºC
   6.13. 1.5 ml microfuge tubes
   6.14. 2X Laemmli Sample buffer, BIO-RAD catalog #161-0737 with added ß mercaptoethanol
   6.15. Precision Plus Protein Kaleidoscope Ladder, (BioRad catalog # 161-0375)
   6.16. Gel loading pipette tips (optional)
   6.17. 10 ml syringe and 21G2 needle

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6.18. Ice bucket and ice
6.19. Affinity chromatography purified rAAV-GFP viral particle

7. Procedure:

7.1. Prepare Samples.

Note: rAAV viral particle 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.
7.1.3. Label one microfuge tube for each sample.
7.1.4. For each sample to be analyzed:

7.1.4.1. Prepare the sample using a sample volume of up to 20 µl. If the sample volume needed is less than 20µl add enough Ultra-pure 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.

7.1.4.2. 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 tabletop 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 Ultra-pure 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 Ultra-pure 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.

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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 2.5µl of the Protein Ladder

7.3.16.2. Load each sample well with the with the total prepared sample volume, (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, blackjack 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 70-90 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. Perform all steps in a single clean staining tray (plastic or glass) with constant gentle shaking

7.5.2. 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

7.5.3. 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.

7.5.4. Carefully open the cassette, the gel will be attached to one of the plates. Discard the plate that is not holding the gel.

7.5.5. 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 Ultra-pure water.

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7.5.6. 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 Ultra-pure water.

7.5.7. Allow the gel to wash in the for 5 minutes on a rotary platform. Replace the Ultra-pure water with 20 ml of fresh Ultra-pure water and repeat this 5-minute wash.

7.6. Stain the gel using silver staining kit
Fixing, Ethanol and Stop solutions (used in steps may be prepared in advance. Other solutions must be prepared immediately before use

7.6.1. Avoid using metal utensils throughout the procedure. Use a clean, plastic spatula or gloved hands to manipulate the gel. When using gloved hands, touch the gel only at the edges to avoid depositing protein on the surface, which may cause background.

7.6.2. Drain the water from the container holding the gel and add approximately 25 ml of fixing solution. Incubate for 15 minutes. Replace the fixing solution with fresh 25 ml fixing solution and incubate for 15 minutes.
NOTE: GEL MAY BE KEPT IN FIXING SOLUTION OVERNIGHT WITHOUT AFFECTING STAIN PERFORMANCE

7.6.3. Drain the fixing solution and wash the gel with 25 ml of 10% ethanol, incubate for 5 minutes. Replace ethanol solution and repeat the wash step

7.6.4. Drain the wash solution from the container and add 25ml of ultrapure water and incubate for 5 minutes. Replace the ultra-pure water with fresh ultrapure water and repeat the 5-minute wash

7.6.5. During the second 5 minutes wash step, prepare Sensitizer working solution by mixing 50µl of sensitizer with 25ml water in a clean sterile 50 ml conical tube (**Note**: **must prepare immediately before use)**

7.6.6. Drain ultrapure water and add 25ml of Sensitizer working solution and incubate exactly for one minute.

7.6.7. Drain the sensitizer working solution and add 25ml of ultra-pure water and incubate for 1 minute. Replace the ultra-pure water with fresh water and repeat 1 minute incubation.

7.6.8. Prepare Stain working solution by mixing 500µl of enhancer and 25ml of Stain in a clean sterile 50ml conical tube

7.6.9. Drain the ultra-pure water and add 25ml of prepared stain working solution and incubate for 30 minutes
NOTE: GEL MAY BE INCUBATED OVERNIGHT IN STAIN WORKING SOLUTION WITHOUT AFFECTING STAIN PERFORMANCE)

7.6.10. Prepare Developer Working Solution by mixing 500µl of silver stain enhancer and 25ml of silver stain developer solution in clean sterile 50ml conical tube

7.6.11. Drain the Silver stain working solution. Quickly wash gel with two changes of ultrapure water for 20 seconds each

7.6.12. Immediately add 25ml Developer working solution and incubate until protein band appears (approximately 2 minutes 23 seconds)
NOTE: Protein bands will begin to appear within 30 seconds and then continue to develop. Between 2- and 3-minutes protein detection vs. background is optimal. After 3 minutes, lane background signal may increase to undesirable levels

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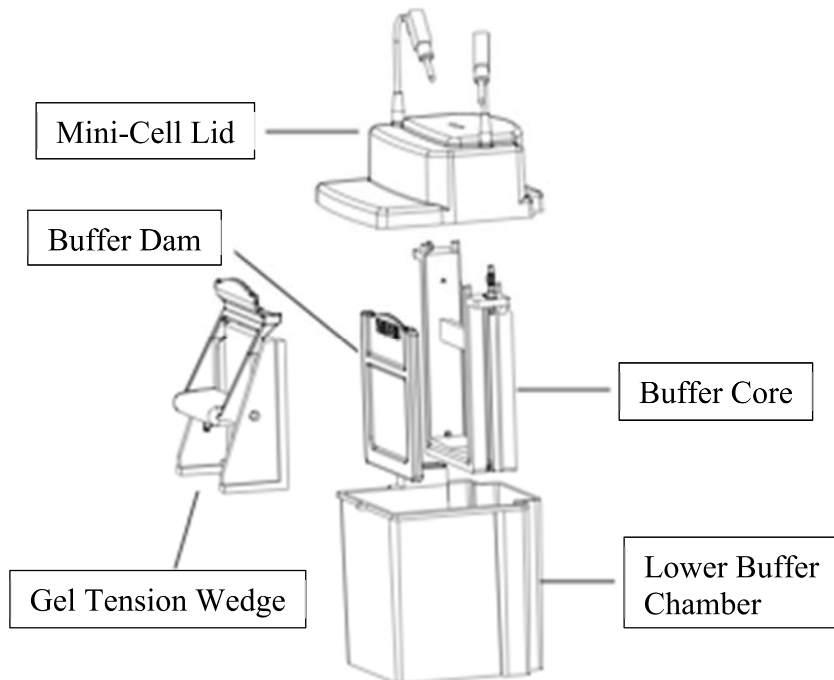
7.6.13. When the desired band intensity is reached replace, Developer working solution with prepared 5% acetic acid (stop solution). After 30 seconds replace the stop solution with fresh stop solution and incubate for 10 minutes.

7.6.14. The stained gel can now be imaged and analyzed

8. Attachments

8.1. Gel Box



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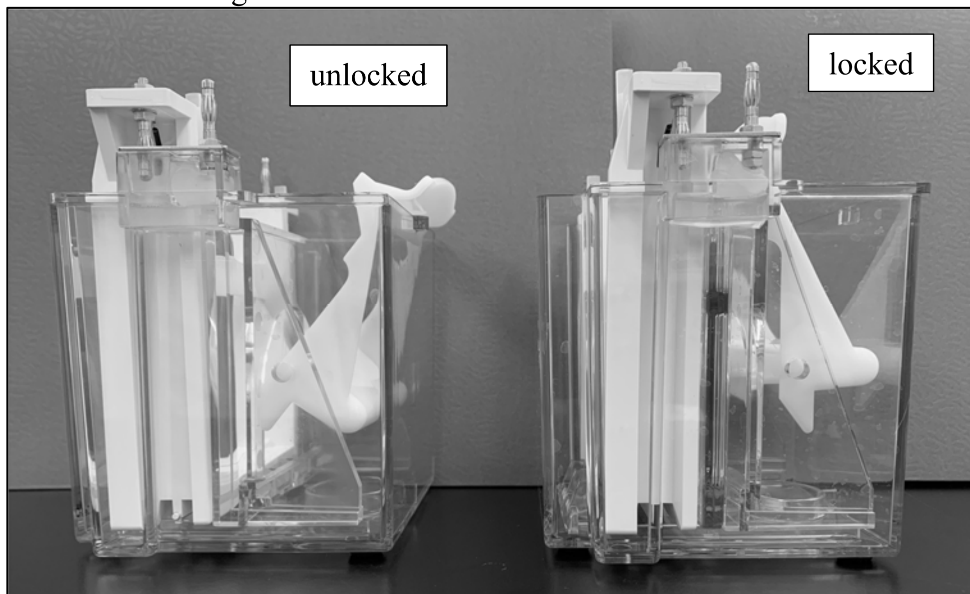
8.2. Gel Tension Wedge Position



9. History:

Revision Number	Effective date	Preparer	Description of Change
0	18JULY23	Hetal Doshi	Initial release