



Vü Imaging System

APPLICATION NOTES




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1 SDS-PAGE Procedure

1. Prepare SDS-PAGE gel of your choice.
2. Allow all reagents stored in the cold, to warm up to room temperature before using it.
3. Follow the manufacturer's instructions for assembling the SDS-PAGE apparatus.
4. Prepare your samples according to your protocol.
5. Prepare electrophoresis buffer and pour the buffer into electrophoresis tank.
6. Load Protein Marker and the samples into designated well.
7. Run the gel at your desired voltage/current until the blue dye front is 0.5 – 2 cm from the bottom of the gel.
8. Once the run is completed, disassemble the gel cassette carefully and place the gel in a tray of 1X Transfer buffer.

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2 Western Blot Procedure

1. Follow the manufacturer's instructions for assembling the Western blot apparatus.
2. Be aware if your selected Protein Binding Membrane needs pre-treatment.
3. Prepare ice-cold 1X Transfer buffer.
4. In a suitable tray, soak 2 sheets of filter paper, 2 pieces of fiber pad, and the Protein Binding membrane in 1X Transfer buffer.
**Number of filter paper and fiber pad depends on manufacturer's instructions.*
5. Open the plastic blot holder and place it in a suitable tray with the dark side or Cathode (-) side down.
6. Place one fiber pad on the cassette, followed by one sheet of filter paper. Ensure no air bubbles are trapped in between.
**Number of filter paper and fiber pad depends on manufacturer's instructions.*
7. Pick up the gel carefully and place it onto the filter paper. Ensure no air bubbles are trapped in between.
8. By using the forceps, place the Protein Binding Membrane onto the gel. Ensure no air bubbles are trapped in between.
9. After that, place another piece of filter paper then follow by the fiber pad, and close the cassette.
**Number of filter paper and fiber pad depends on manufacturer's instructions.*
10. Transfer the cassette to the Western blot apparatus and pour the 1X Transfer buffer into the tank.
11. Transfer the proteins according to your desired voltage/current.
12. Once the transfer is completed, open the cassette carefully and remove the Protein Binding Membrane with forceps.
13. Place the Protein Binding Membrane in a suitable tray with 1X Washing Buffer.
14. Wash the Protein Binding Membrane twice for 5 minutes each with 1X Washing Buffer (enough volume to cover the membrane), and place the tray on a shaker with gentle shaking.
**Incubation time depends on your protocol.*
15. Discard the 1X Washing Buffer and add 1X Blocking Buffer (enough volume to cover the membrane) into the tray. Incubate at room temperature for 30 – 60 minutes with gentle shaking. Or, this can be left at 4°C overnight.
**Incubation time depends on your protocol.*

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16. Prepare the Primary antibody according to manufacturer's instructions.
17. Discard 1X Blocking Buffer and add the Primary antibody to the Protein Binding Membrane. Then, incubate for 30 – 60 minutes at room temperature with gentle shaking.
**Incubation time and temperature depends on your protocol.*
18. Discard the Primary antibody and wash 3 times for 10 minutes each with 1X Washing Buffer.
**Incubation time depends on your protocol.*
19. Prepare the Secondary antibody according to manufacturer's instruction.
20. Discard the 1X Washing Buffer and add the Secondary antibody to the Protein Binding Membrane. Then, incubate for 30 – 60 minutes at room temperature with gentle shaking.
**Incubation time and temperature depends on your protocol.*
21. Discard the Secondary antibody and wash 3 times for 10 minutes each with 1X Washing Buffer.
**Incubation time depends on your protocol.*
22. Discard 1X Washing Buffer and add appropriate volume and type of Substrate to the Protein Binding Membrane.
23. Incubate for 5 minutes at room temperature with gentle shaking.
**Incubation time and temperature depends on your protocol.*
24. The membrane is ready to be visualized with Imaging System.

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3 How to Capture Image of Chemiluminescence Blot

1. Ensure the Vü system is switched on and the LED on the front of the system has turn Green (indicates that the system is ready).
2. Ensure that the Vü system is connected to your computer and the Vü software is opened.
3. Position the Black blot cover (FACE UP) on a flat surface.

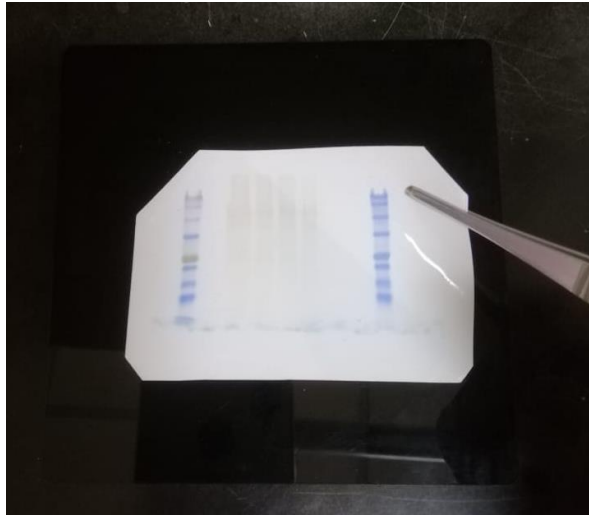


4. Remove the blot from the storage container and drain excess liquid from the blot.

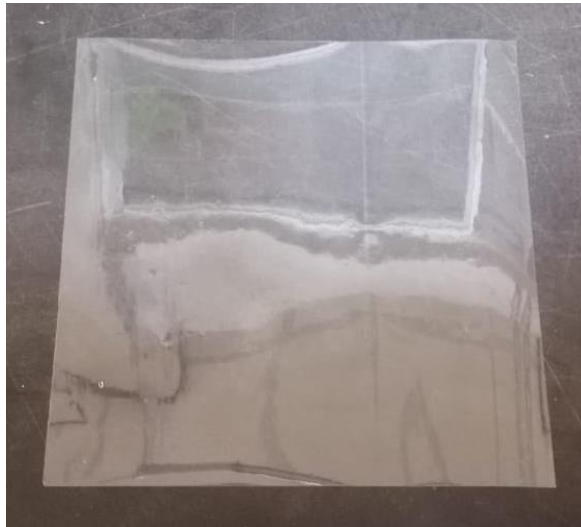


5. Ensure the blot is moist and doesn't have excess liquid on it.

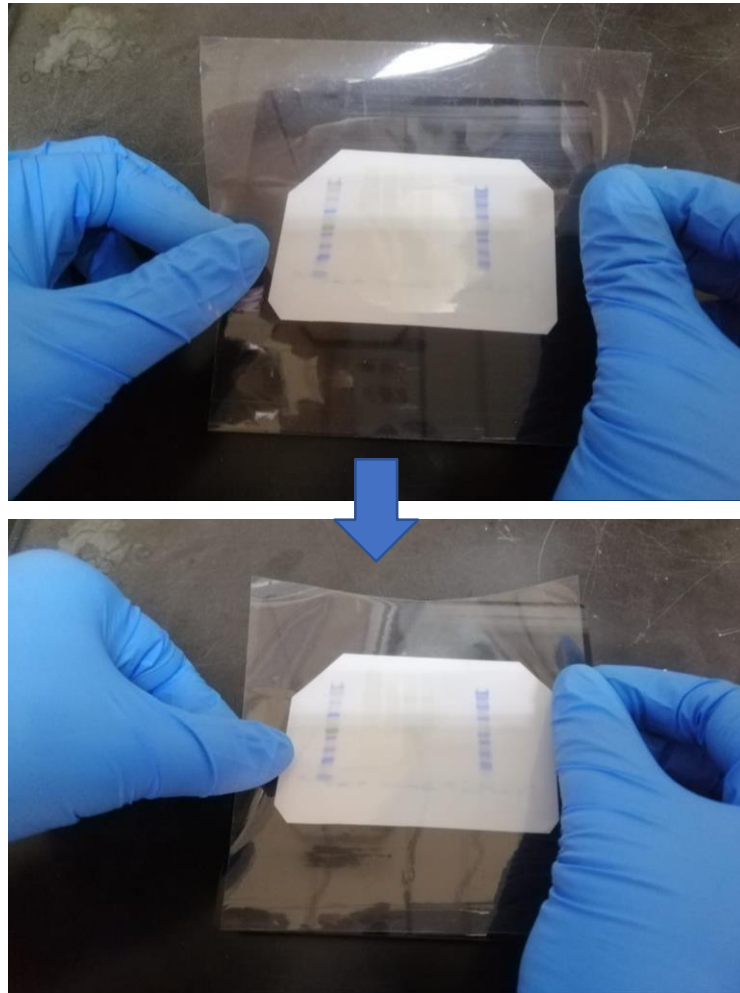
6. Place the blot (FACE UP) on the Black blot cover.



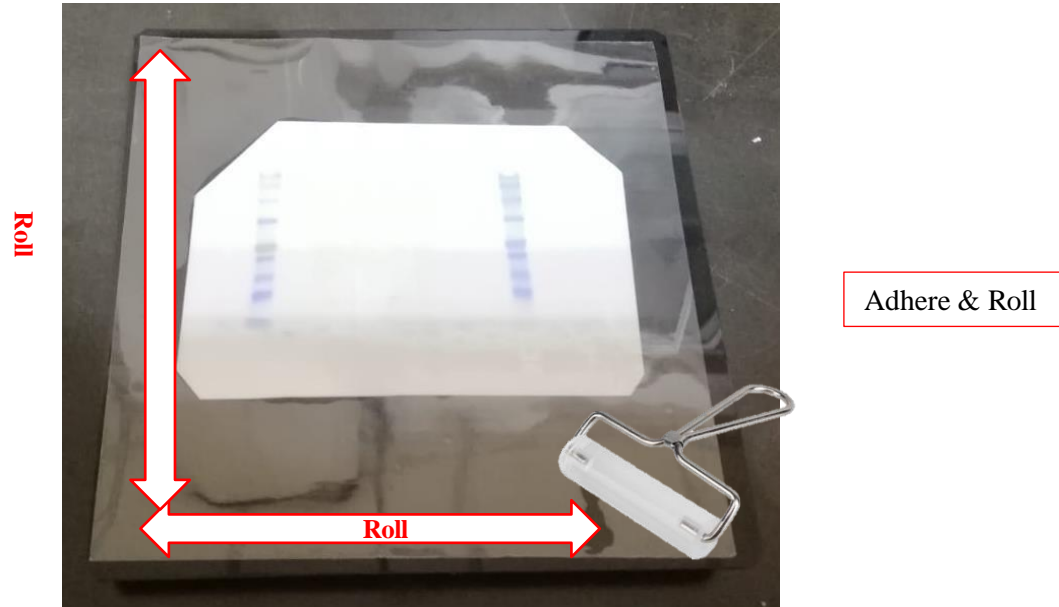
7. Get ready a piece of blot plastic wrap beforehand. The size shouldn't larger than the Black blot cover.



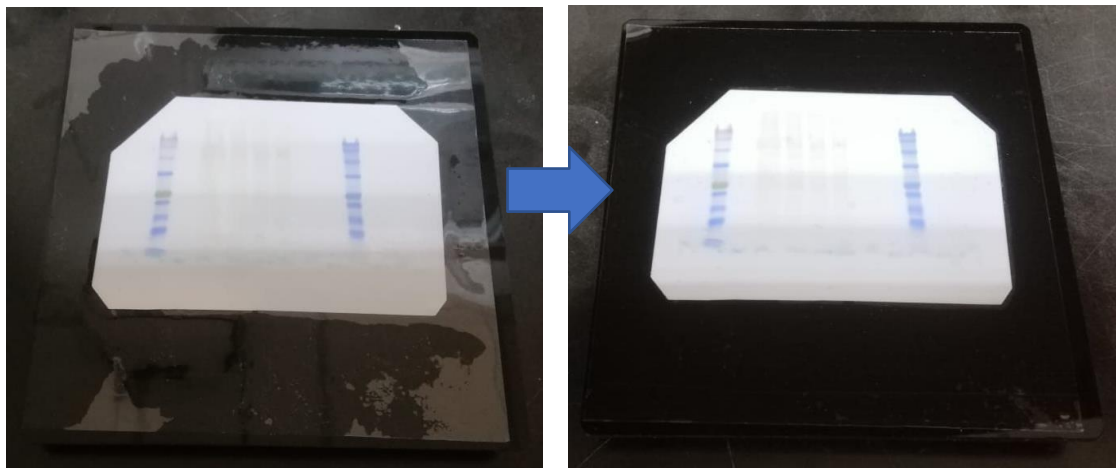
8. Place the blot plastic wrap on top of the blot. Ensure there are no air bubbles in between the plastic wrap and the blot.



9. Once the blot plastic wrap is adhered to the blot and Black blot cover, use a roller to roll **all** over the blot plastic wrap. This is to ensure the blot is flat and also to remove any air bubbles that are trapped in between the blot plastic wrap and the blot.



10. Once you have finished rolling the blot plastic wrap, you should observe no air bubbles in between the blot plastic wrap and the blot, excess liquid has moved to the sides of the blot, and the blot plastic wrap is fully adhered to the Black blot cover.

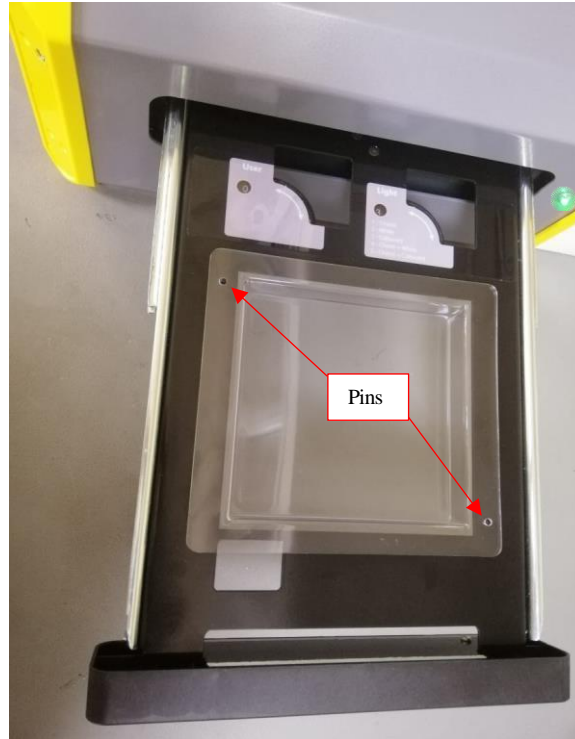


11. Pull out the drawer from the front of the unit as far as you can. It will reach a “stop” point.

12. Select one of the Light options at Vü-C to capture an image:

- i. Chemi (Chemiluminescent)
- ii. White
- iii. Coloured
- iv. Chemi + White
- v. Chemi + Coloured

13. Place the Clear tray into the drawer and make sure it is located on the two “pins”. Failure to do this could result in the drawer getting jammed.



14. Now, place the Black blot cover (with the blot and blot plastic wrap) onto the Clear tray.



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15. Once the Black blot cover is positioned onto the Clear tray, push the drawer all the way in.

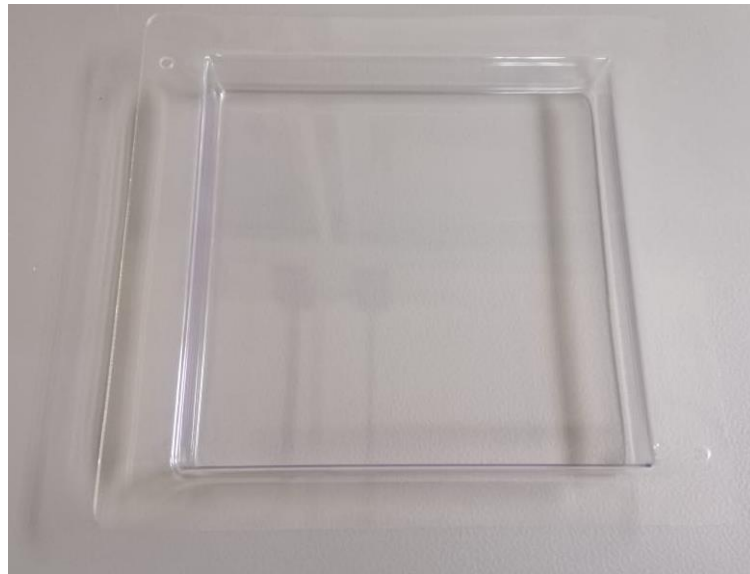


16. The machine will start automatically.

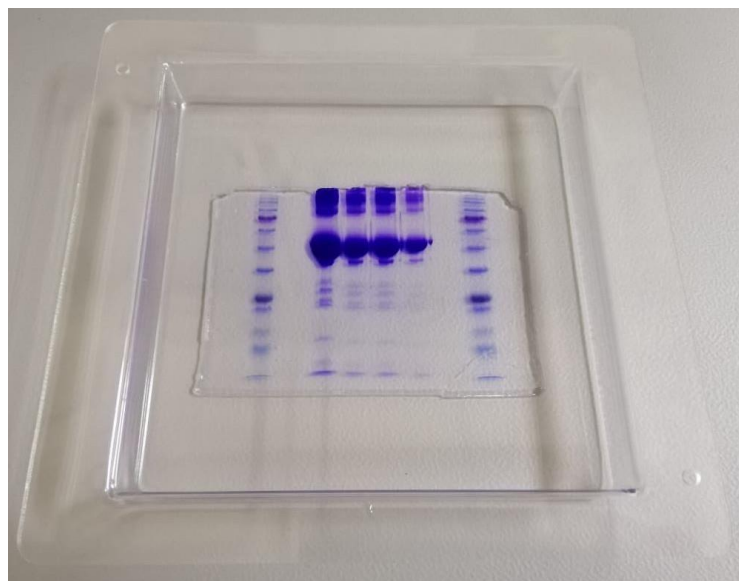
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4 How to Capture Image of SDS-PAGE Gel on Vü-Chemiluminescence

1. Ensure the Vü system is switched on and the LED on the front of the system has turn Green (indicates that the system is ready).
2. Ensure that the Vü system is connected to your computer and the Vü software is opened.
3. Get ready the Clear tray on a flat surface.



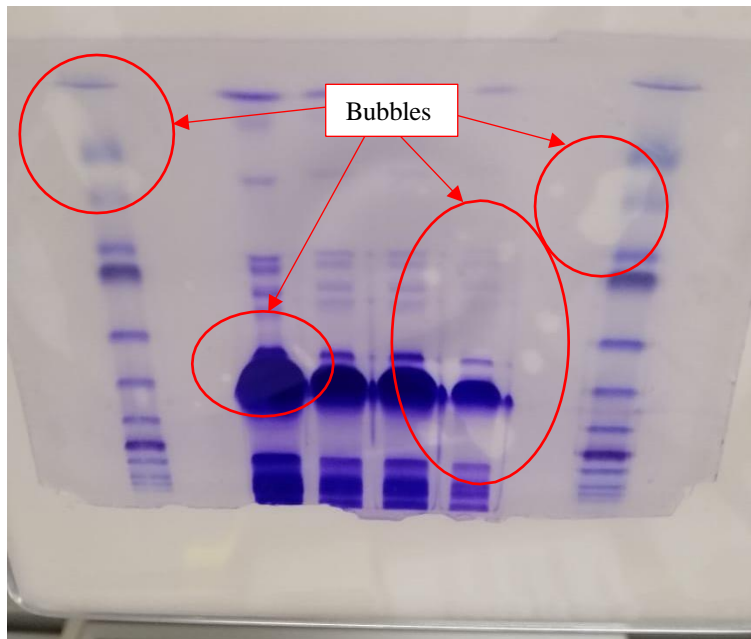
4. Place the SDS-PAGE gel onto the Clear tray. Ensure the gel is moist but doesn't have excess liquid on it and ensure no bubbles are trapped in between the gel and the tray.



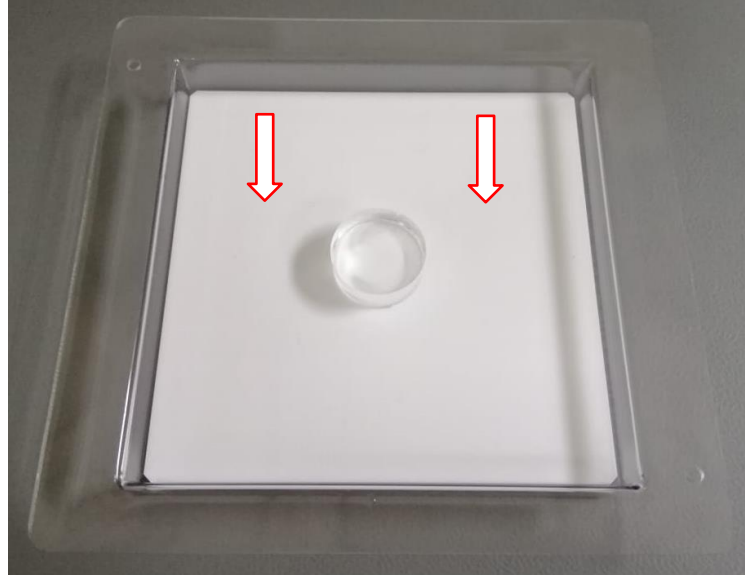
5. Apply the White cover blot onto the gel.



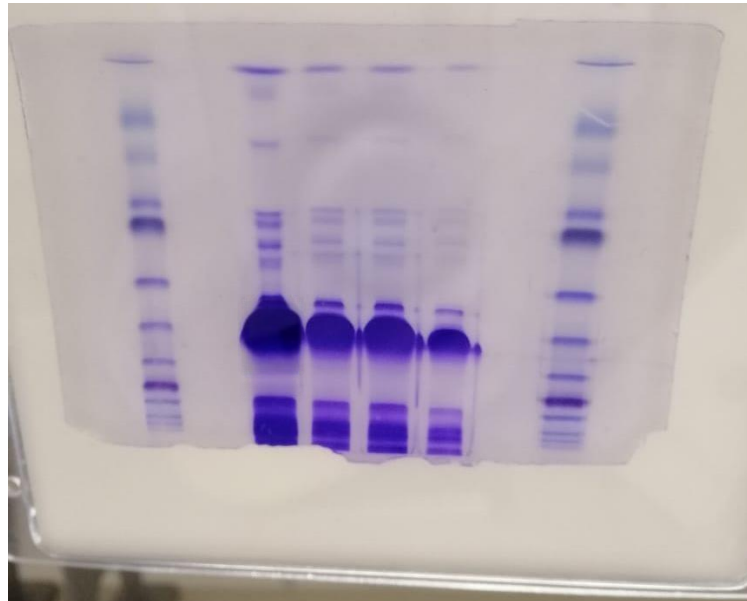
6. Now, look from the bottom of the tray and see if there is any bubble. Be careful not to overturn the tray.



7. If bubbles are observed at this point, try to remove the bubbles by applying some pressure at any area of the White blot cover.

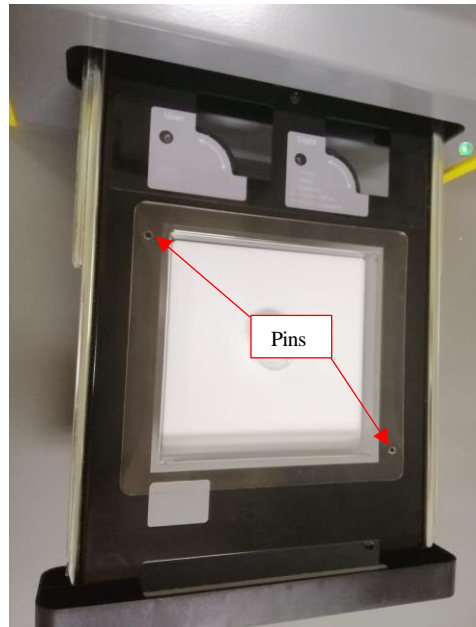


8. After applying some pressure to move the bubbles out of the gel, the gel is ready for imaging.

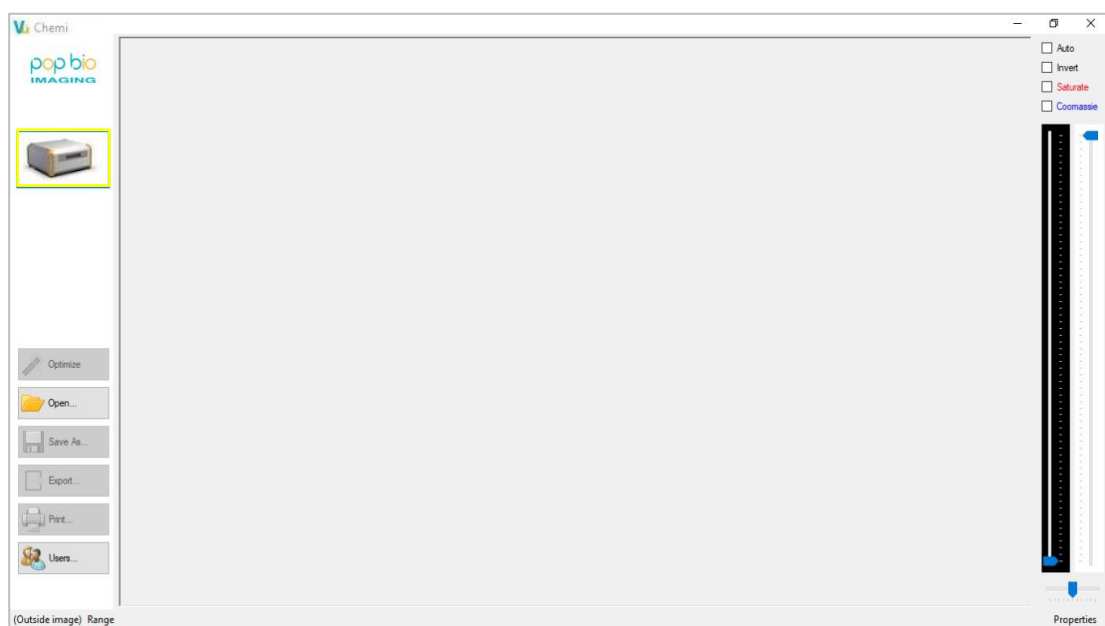


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9. Pull out the drawer from the front of the unit as far as you can. It will reach a “stop” point.
10. Place the tray (with the gel) onto drawer frame and make sure it is located on the two “pins”. Failure to do this could result in the drawer getting jammed.

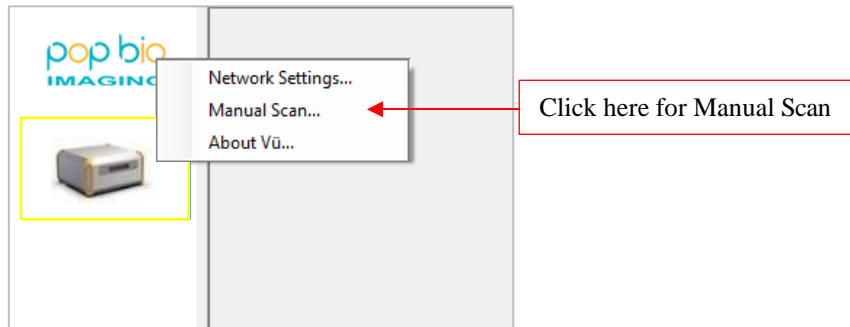


11. Don't close the drawer just yet.
12. Go to the Vü software that you have opened and connected to your PC (should see a screen as shown below).



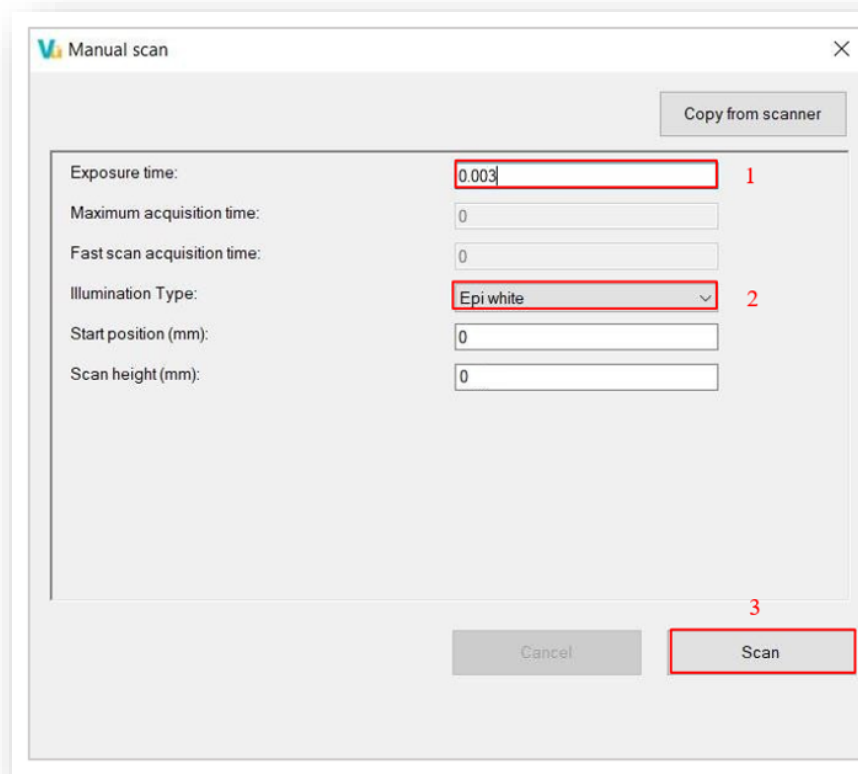
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13. Click the Pop Bio Imaging logo on the top left corner of the screen. Then, select Manual Scan.



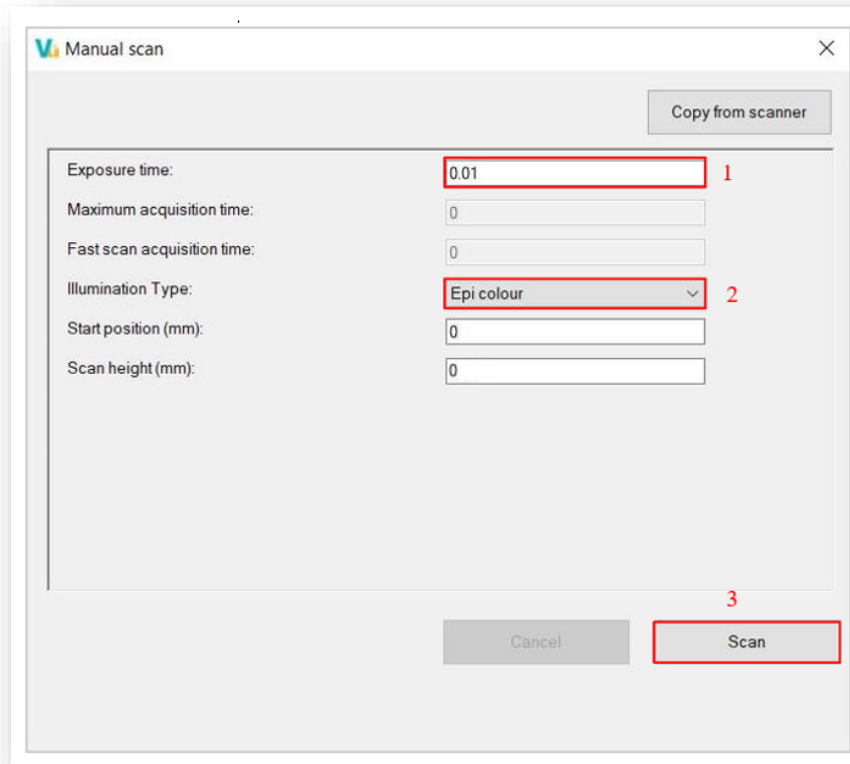
14. To capture **Black & White** image of the gel, do the following:

- i. Change Exposure time: **0.003**,
- ii. Change Illumination Type: **Epi white**,
- iii. Click **Scan**.



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15. To capture **Colour** image of the gel, do the following:
- i. Change Exposure time: **0.01**,
 - ii. Change Illumination Type: **Epi colour**,
 - iii. Click **Scan**.



16. Once you have done the settings for Manual Scan, you can now push the drawer all the way in and the machine will start automatically.