

SEMI-DRY BLOTTING

BENCHNOTES

Semi-Dry Blotting of Proteins

The following procedure is formulated for applications with SDS-PAGE gels. If a high molecular weight protein that is greater than 100,000 DALTONS is being semi-dry blotted, the addition of 0.1% SDS to the cathode buffer may facilitate the transfer.

Solutions

| | |
|------------------------------|---|
| Anode Buffer #1 | 0.3 M Tris pH 10.4 20% Methanol |
| Anode Buffer #2 | 0.025 M Tris pH 10.4 20% Methanol |
| Cathode Buffer | 0.025 M Tris pH 9.4 0.4 M Amino Caproic Acid 20% Methanol |
| Tris-Glycine-Methanol Buffer | 25 mm Tris pH 8.3 192 mm Glycine 20% Methanol |

Recommended Current Requirements for Semi-Dry Blotting of Proteins

| Current Density | Trans-Unit | Time Limit |
|------------------------|------------|---------------|
| 0.8 mA/cm ² | 1-6 | 1-2 hours |
| 2.5 mA/cm ² | 1-6 | 30-45 minutes |
| 4.0 mA/cm ² | 1-6 | 15-30 minutes |

Procedure

1. Presoak the gel in Tris-Glycine-Methanol buffer for 15-30 minutes at room temperature.
2. Precut the filter paper and the solid support membrane the same size as the gel.
3. Presoak the nitrocellulose in distilled water for 5 minutes at room temperature.
4. Place 2 sheets of filter paper soaked with 15-20 ml of Anode Buffer #1 on the anode (red) electrode plate of the Semi-Dry Blotter followed by a sheet of filter paper soaked with Anode Buffer #2.
5. Lay the wet membrane from Step 3 on the filter paper.

6. Carefully lay the pre-soaked gel on the membrane stack without allowing any air bubbles between the gel and the membrane.
7. Lay one sheet of the filter paper soaked with cathode buffer on the gel.
8. If stacking another Trans-Unit on top of the first one, lay a dialysis membrane (presoaked in distilled water and cut to the same size as the filter paper) on the cathode filter paper and then start another stack of the Trans-Unit.
9. Complete the Trans-Unit with 2 sheets of filter paper soaked with the cathode buffer.

Notes

3M filter paper must be very wet but not to the point of having puddles of standing buffer.

It is important for all the members of the Trans-Unit to be exactly the same size or properly masked with plastic wrap to avoid distorted patterns and/or short circuits.

Care must be taken to completely change the ionic composition of the gel before electro-transfer. Insufficient soaking will result in a gel with too high an electrical conductivity, resulting in overheating and/or incomplete transfer.

Up to 6 gels may be transferred simultaneously by stacking Trans-Units if they are separated by sheets of low-porosity dialysis membrane. The dialysis membrane will prevent cross-contamination.

References

David, Leonard G., Dibner, Mark D., Batley, Jim F. *Basic Methods in Molecular Biology*.

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Semi-Dry Blotting of DNA

DNA (and RNA) may be easily and rapidly transferred to charged modified nylon membranes by semi-dry electro-blotting. The procedure below is written specifically for DNA blots from agarose gels but is easily adapted to other situations.

Solutions

| | |
|----------------------------|---|
| Denaturizing Solution (DS) | 0.5 M NaOH, 1.5 M NaCl |
| Neutralizing Solution (NS) | 1.0 M Tris HCl pH 8.0 1.5 M NaCl |
| Depurination Solution (DP) | 0.25 M HCl |
| Transfer Solution (TBE) | 0.089 M Tris 0.089 M Boric Acid 0.002 M EDTA pH 8.3 |

Procedure

1. Electrophoresis, staining and photography are performed as usual.
2. Trim away unused portions of the gel. Prepare 8 pieces of Whatman 3M filter paper and one sheet of Nylon 66 **exactly** the size of the trimmed gel.
3. Soak gel twice in 2 times the gel volume of H₂O for 10 minutes (total time — 20 minutes).
4. Soak gel twice in 2 times the gel volume of DS for 30 minutes at room temperature with constant shaking (total time — 1 hour).
5. Soak gel twice in 2 times the gel volume of NS for 30 minutes at room temperature with constant shaking (total time — 1 hour).
6. Soak gel twice in 2 times the gel volume of TBE for 30 minutes (total time — 1 hour).
7. Wet 4 pieces of Whatman 3M paper in TBE and place on the Semi-Dry Blotter anode (red) electrode plate. Be sure there are no air bubbles beneath the paper.
8. Wet the Nylon 66 membrane and place it on top of the wet filter paper. Be sure there are no air bubbles beneath the membrane.
9. Place the treated gel on the membrane, making sure not to trap air beneath it. **Do not** move the gel once it is in place.
10. Cover the gel with 4 layers of 3M filter paper wetted in TBE.

11. Carefully lower the Semi-Dry Blotter cathode (black) electrode into place on top of the assembled Trans-Unit.
12. Make the appropriate electrical connections to a suitable power supply.

Typical run conditions are 0.5 to 3 mA/cm² gel area (5-20 volts depending on gel size).

Run times will vary with DNA size and current density. Typically 30-60 minutes will be required at current densities of 2-3 mA/cm².

Optional Depurination Step

Very high molecular weight DNA (>20kb) may be reduced in size for more efficient transfer. Soak gel twice in 2 times the gel volume of DP solution for 30 minutes prior to denaturation.

It is very important not to let the depurination step proceed too far as the very small fragments produced do not bind efficiently to the membrane.

Notes

3M filter paper must be very wet but not to the point of having puddles of standing buffer.

It is important for all the members of the Trans-Unit to be exactly the same size or properly masked with plastic wrap to avoid distorted patterns and/or short circuits.

Care must be taken to completely change the ionic composition of the gel before electro-transfer (Step 6). Insufficient soaking will result in a gel with too high an electrical conductivity, resulting in overheating and/or incomplete transfer.

Up to 6 gels may be transferred simultaneously by stacking Trans-Units if they are separated by sheets of low-porosity dialysis membrane. The dialysis membrane will prevent cross-contamination.

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Semi-Dry Blotting Troubleshooting

Poor Transfer

- protein still in gel
- increase transfer time
- decrease methanol which decreases binding
- filter paper too dry

Swirls, Missing Bands

- contact of gel to membrane is poor
- excessive fluid or bubbles in sandwich
- gel not equilibrated
- power conditions too high
- membrane pore size too large

Poor Binding

- low grade membrane
- ester contamination
- use 20% methanol or optimal binding
- improper membrane pore size
- use Tween 20 as detergent in wash solutions or none at all. Other types of detergents can remove proteins from the membranes.

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