

Western Blot Technique to Resolve Von Willebrand Factor Multimers and Degradation Fragments from LVAD Plasma

Part I: Electrophoresis and Immunoblotting

Agarose Gel

Gel buffer: 0.1% SDS, 0.375 M Tris-Base
1.0% Agarose = 0.10g agarose per 10mL gel buffer
Prepare 15ml/cassette

Sample Prep

Dilute plasma 1:40 (10 μ l+390 μ l) in 1x LDS sample buffer
Heat at 70°C for 10 minutes

Electrophoresis

Load 15 μ l per lane
Agarose: 60V for 2 hr 30 min
Polyacrylamide: 150V for 1 hr 25 min

Transfer

iBlot, setting P2 for 8 min 30 sec
Block 1 hr in 5% milk-PBS

Probe for vWF

Incubate for 1 hr in 10mL of primary anti-human vWF 1:500 in milk-PBS
Wash 3 times for 5 minutes in ~20ml PBS
Incubate for 1 hr in 10 mL of secondary goat anti-rabbit 1:3,000 in milk-PBS
Wash 3 times for 5 minutes in ~20ml PBS

Image

Develop with 2mL HRP substrate
Image at high resolution at 10 second intervals, and save images as TIFF files

Probe for Albumin

Wash 3 times for 5 minutes in ~20ml PBS
Incubate for 20 minutes in 10 mL of HRP-conjugated anti-human albumin 1:10,000 in milk-PBS
Wash 3 times for 5 minutes in ~20ml PBS
Image for <10 seconds

Part II: Agarose/Polyacrylamide Transfer iBlot Gel to PVDF Membrane

1. Cut all edges from agarose/ polyacrylamide gel and mark top corner
2. Open "bottom" portion of iBlot packet
3. Place gel onto the top of the iBlot layers
4. Wet a blotting sheet with water and place on top of gel
5. Place copper "top" sheet of iBlot packet with gel facing down
6. White foam sheet goes on the top of the iBlot machine with metal on the top right side
7. Close the machine and run at P2 for 8:30 minutes

5% Milk buffer blocking

1. First block
 - a. 1/500 dilution in 5% milk
 - i. $(1)(x) = (1/500)(10\text{ml})$
 - ii. .02ml
 - iii. 20 μ l antihuman + 10ml milk
2. Second block
 - a. 1/3000 dilution in 5% milk
 - i. $(1)(x) = (1/3000)(10\text{ml})$
 - ii. .003ml
 - iii. 3.3 μ l anti-rabbit + 10ml milk
3. Albumin
 - a. 1/10000 dilution in 5% milk
 - i. $(1)(x) = (1/10000)(10\text{ml})$
 - ii. .001
 - iii. 4 μ l anti-human albumin + 40ml milk