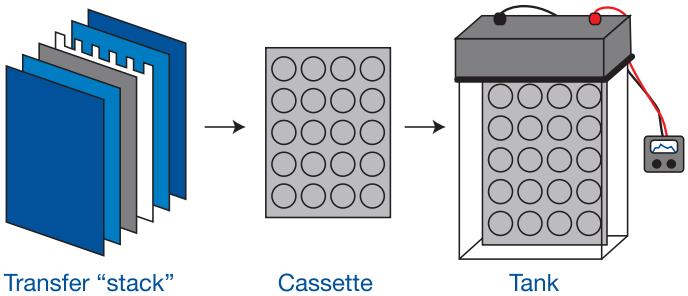
Wet or Dry?
Which Type of Transfer is Best for Your Protein?

To perform a Western blot, proteins are separated according to molecular weight by electrophoresis through a polyacrylamide gel. The proteins are then transferred from the gel onto a solid membrane support for subsequent immunodetection by protein-specific antibodies. Transfer occurs by applying an electric field to the gel, which induces migration of charged proteins out of the gel towards the direction of an oppositely charged electrode. In most systems, negatively charged proteins migrate out of the gel towards the positively charged electrode.

Transfers can be performed by [wet] transfer, in which transfer occurs while the gel and membrane are submerged under a buffer in a tank apparatus, or under semi-dry conditions, in which transfer occurs between two electrode plates. The type of transfer apparatus used can affect the efficiency of transfer of proteins out of the gel and retention of proteins on the membrane. The benefits and drawbacks of each system should be taken into consideration when optimizing transfer conditions for a protein.

Wet transfer

To perform a wet transfer, a transfer "stack" is built consisting of a transfer sponge, filter paper, membrane, gel, filter paper and a second transfer sponge. Prior to assembling the stack, all parts (including the gel) are equilibrated in transfer buffer. The stack is placed inside a plastic cassette that is then submerged in transfer buffer within a tank. Electricity is applied to the tank through attached electrodes and the proteins migrate out of the gel onto the membrane following the current applied through the transfer buffer. Either wire or plate electrodes are contained within the tank. Wet transfer is useful for most general Western blotting applications.



Benefits of wet transfer

Using a wet transfer apparatus, high intensity (1-2 hour) or lower intensity (overnight) transfers can be performed. This allows for optimizing transfer conditions for each individual protein. Transfer times can be shortened to prevent transfer of low molecular weight proteins through the membrane while longer transfer times can be used to promote complete migration of high molecular weight proteins out of the gel.

Drawbacks to wet transfer

The transfer process generates heat, which can decrease the resistance of the transfer buffer resulting in inconsistent transfer across the gel. High heat can also result in breakdown of the gel itself. To prevent heating, transfer buffer should be pre-chilled prior to use. In addition, the transfer buffer should be kept cold during transfer. Long transfers are often

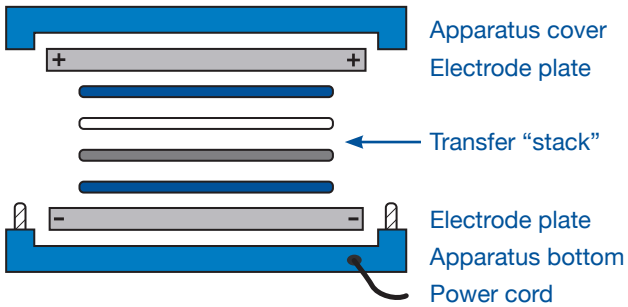
Wet transfer

Advantages	Disadvantages
• Flexible: multiple transfer conditions can be adjusted easily • Multiple buffers to optimize transfer • Transfers broad molecular weight range at one time • Extended transfer possible • Can be used for quantitative Westerns	• Heating of buffer can interfere with transfer • Cooling mechanism and/or cold room space required during transfer • Large volumes of transfer buffer are required

performed in a cold room to aid in keeping the buffer cool. Additionally, self-contained ice blocks can be placed within the tank and are usually supplied with the apparatus. Alternatively, external-cooling mechanisms can be used to control heating.

Semi-dry transfer

For semi-dry transfer, the stack (consisting of filter paper, the gel and membrane all pre-equilibrated in buffer) is built directly on the bottom electrode plate of the apparatus. The cover of the apparatus (which contains the second electrode plate) is placed firmly over the stack and electricity is applied through the electrodes. High electric field strengths are achieved due to the minimal distance between the electrodes (the thickness of the stack) resulting in rapid (<1h) transfer.



Benefits to semi-dry transfer

Semi-dry transfers are fast and easy and require little buffer. Transfer of more difficult to transfer proteins can be optimized by using a discontinuous buffer system, a feature unique to semi-dry transfer systems. In a discontinuous buffer system, the filter paper placed on the anode side of the stack is soaked in a different buffer from the filter paper placed on the cathode side of the stack. This can increase migration of the protein out of the gel while also promoting better retention on the membrane.

Drawbacks to semi-dry transfer

Semi-dry transfer systems have less flexibility and it can be difficult to transfer both high and low molecular weight proteins. Low molecular weight proteins can transfer through the membrane due to the high intensity blotting conditions while high molecular weight proteins may not efficiently transfer out of the gel due to decreased transfer times. However, transfer of high molecular weight proteins can be improved by using the discontinuous buffer system. Transfer times cannot be extended when using semi-dry transfer, as there is limited buffering capacity. In addition, the gel can dry out if insufficient buffer is used.

Semi-dry transfer

Advantages	Disadvantages
• Transfer is rapid • Can use discontinuous buffer system to optimize transfer of proteins • Little buffer is required • Easy to set up • Good for performing large numbers of blots analyzing the same protein	• High intensity field strength may cause low molecular weight proteins to migrate through membrane • Difficulty in transferring high (>120 kDa) molecular weight proteins • Not recommended for quantitative Westerns • Gel can dry out

Tips for successful transfers

- Take care when preparing transfer buffers; small inconsistencies can affect transfer
- Use high quality, reagent grade methanol when preparing buffers; impurities in methanol can decrease transfer efficiency
- Never dilute the transfer buffer
- Do not adjust pH of the transfer buffer
- For optimum transfer, do not reuse transfer buffer
- Make sure all equipment is clean
- Use a thinner gel (0.5-0.75 mm thickness)
- If using a PVDF membrane, pre-wet the membrane in 100% methanol prior to equilibration in transfer buffer
- Equilibrate transfer pads, filter paper, membrane and gel for at least 15 minutes in buffer(s)
- Remove all air bubbles and creases between each layer in the stack
- Ensure the stack is firmly held in place and pressure is applied evenly over the entire surface of the stack
- Ensure that electrodes are connected and free of debris and damage (plate electrodes)
- Make sure the current is applied correctly and proteins will migrate towards the membrane

- Use pre-stained molecular weight standards to help monitor transfer
- Stain the blot with a reversible total protein stain (e.g. Ponceau S) to check quality of transfer
- For difficult to transfer proteins, adjust methanol and SDS concentrations. SDS promotes migration of proteins out of the gel but can inhibit membrane binding. Methanol increases retention of proteins on the membrane but can hinder migration out of the gel.

Wet transfer buffers

Towbin Transfer Buffer

- Standard wet transfer buffer
- SDS (0.025-0.1%) can be added to facilitate transfer of proteins

25 mM Tris, pH 8.3
192 mM glycine
20% methanol
+/- SDS

CAPS (3-[cyclohexylamino]-1 propane sulfonic acid) Buffer

- For blotting basic proteins
- For blotting prior to N-terminal sequencing

10 mM CAPS, pH 11
10% methanol

Dunn Carbonate Buffer

- For higher efficiency transfer of basic proteins
- Can be used to enhance ability of antibodies to recognize some antigenic sites

10 mM NaHCO3
3mM Na_2CO_3, pH 9.9
20% methanol

Semi-dry transfer buffers

Bjerrum Schafer-Nielsen Buffers

- Standard semi-dry transfer buffer based on Towbin

48 mM Tris, pH 9.2
39 mM glycine
20% methanol

Discontinuous Tris-CAPS Buffer System

- Uses two different buffers to enhance transfer of proteins
- The filter paper assembled on the membrane side (anode) of the blot contains methanol
- The filter paper on the gel side (cathode) of the blot contains SDS

60 mM Tris, pH 9.6
40 mM CAPS
+ either 15% methanol or 0.1% SDS

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