

# Enhanced Protein Transfer, Including High Molecular Weight Proteins

Western blot analysis of proteins is a routine and commonly used technique in research laboratories, with 3 major drawbacks. The first is the amount of time taken to transfer the proteins to a protein binding membrane; the second is the variable efficiency of the transfer and the third is problems in transferring high molecular weight proteins. Other minor drawbacks also exist with the Western blotting technique and these include overheating of the apparatus, shorting out of power packs due to excess current and the messy assembling of transfer sandwiches.

SWIFT™ transfer pads alleviate the above issues with Western blotting when incorporated in the Western blot sandwich. Each SWIFT™ transfer pad can reduce transfer time by up to 50%, while consistently producing high efficiency transfer. The SWIFT™ transfer pad technology prevents overheating and power shortages by allowing lower chemical concentrations in the transfer buffers, without affecting transfer efficiency. The SWIFT™ transfer pad technology combines the simplicity of semi-dry sandwich assembly with the improved efficiency of wet blot transfers, reducing the need for assembly in large tanks of buffer.

## APPLICATION

15µg mouse liver lysate was resolved on a 4-20% SDS polyacrylamide gel. Following electrophoresis, the proteins were transferred to a PVDF membrane using a standard or the Swift Western Transfer pad.

### Standard Protocol

The PVDF membrane was first rinsed in methanol for 15 seconds and the Western blot sandwich was assembled using a standard Western blotting buffer (25mM Tris, 192mM glycine, 10% methanol).

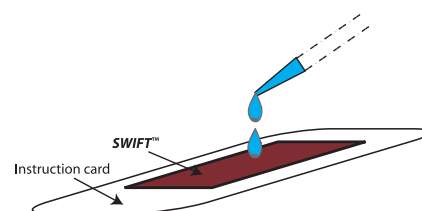
The protein was transferred to the membrane for 30 minutes at a 200mA fixed current.

### Swift™ Western Transfer Pad Protocol (Figure 1)

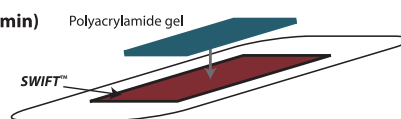
The PVDF membrane was first soaked in 100% methanol for 1-2 minutes then rinsed 2-3 times with deionized water. The Swift™ Template Card was placed on a flat surface and a SWIFT™ Western Transfer Pad placed on the Template Card. Diluted SWIFT™ Western Buffer was added to the top of the pad. The gel was carefully placed on top of the SWIFT™ Western Transfer Pad, ensuring no air bubbles were formed. The SWIFT™ Western Transfer Pad and gel were incubated for 5 minutes at room

temperature. During the incubation, the PVDF membrane was equilibrated in diluted SWIFT™ Western Buffer. The membrane was then laid on top of the gel, ensuring no air bubbles are formed. A second SWIFT™ Western Transfer Pad was placed on top of the membrane and the entire sandwich was placed into a transfer cassette that included fiber pads soaked in diluted SWIFT™ Western Buffer. The assembled transfer cassette was inserted into a Western transfer module with diluted SWIFT™ Western Buffer and run it for 30-60 minutes at 200mA fixed current.

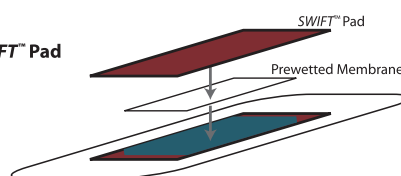
#### 1. Add Transfer Buffer



#### 2. Place Gel & Incubate (5 min)



#### 3. Place Membrane & SWIFT™ Pad



#### 4. Transfer to Blotting Unit & Transfer

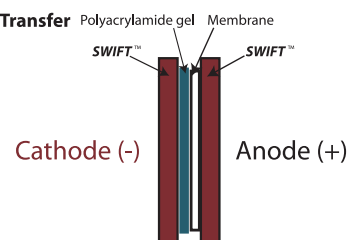
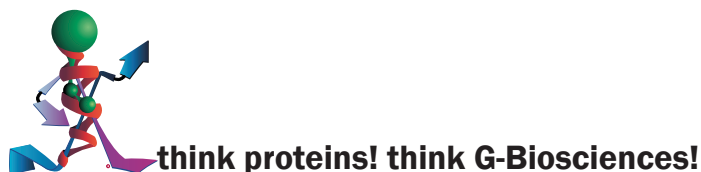


Figure 1: Assembly of the Western blot transfer sandwich using Swift™ Western transfer pads.



Following transfer, the amount of transferred protein was visualized with G-Biosciences' BLOT-FastStain™.

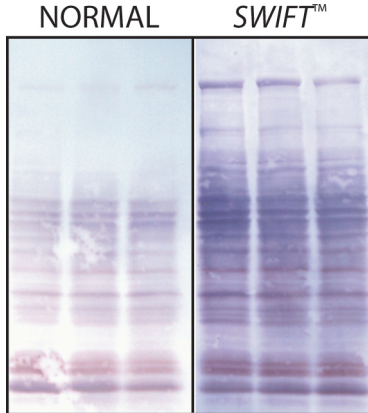


Figure 2: Increased efficiency in protein transfer by SWIFT™ transfer pad. 15µg mouse liver lysate was transferred normally (left) or with a SWIFT™ transfer pad (right) for 30 minutes and the resulting membranes were stained for protein with BLOT-FastStain™.

## RESULTS

The above image clearly shows a significantly higher amount of protein detected on the PVDF membrane when the Swift™ Western Transfer pads were used.

In addition to the higher levels of proteins, there is also a uniform transfer of proteins, which is evident when the high molecular weight proteins are compared between the blots.

Transfer times are normally one hour or longer; this probably accounts for the poor transfer under normal conditions, where transfer was only 30 minutes. In the case of the Swift™ Western Transfer pads, 30 minutes is ample time for high quality transfer.

## CONCLUSION

Swift™ Western Transfer pads offer several benefits compared to standard Western blot procedures:

- Efficient & uniform protein transfer
- Shorter transfer time
- Improved high molecular weight protein transfer

## ORDERING INFORMATION

VWR Cat. No.	Description	Size
78000-006	SWIFT™ Western (Mini)	For 10 Blots
78000-008	SWIFT™ Western (Mini), nitrocellulose	For 10 Blots
78000-010	SWIFT™ Western (Mini), PVDF	For 10 Blots
78000-012	SWIFT™ Western (Medi)	For 5 Blots
78000-014	SWIFT™ Western (Medi), nitrocellulose	For 5 Blots
78000-016	SWIFT™ Western (Medi), PVDF	For 5 Blots
82021-410	BLOT-FastStain™	25 Blots
82021-808	Mouse Normal Liver GenLysate™	150µg

**VWR**  **1.800.932.5000**  
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