One Less Step To Western Blot Development

SWIFT™ Western Diluent is a new generation Western blotting reagent developed by proteomics experts at G-Biosciences. The single reagent SWIFT™ Western Diluent simplifies protein detection by Western blotting and reduces the overall time spent on Western blot development. Traditional Western blotting requires a blocking step to eliminate non-specific binding and the majority of published protocols recommend incubating the blot membrane in blocking solutions from 1hr to overnight. SWIFT™ Western Diluent has been developed to eliminate the time consuming blocking step (Figure 1).

AIM

G-Biosciences aim was to re-evaluate the Western blot development procedure with the aim of simplifying the process. G-Biosciences was able to develop the single reagent SWIFT™ Western Diluent that eliminates the blocking step altogether and save hours from Western blotting. This application note test the performance of SWIFT™ Western Diluent with respect to Western blot development. The effects of SWIFT™ Western Diluent on primary and secondary antibody interactions and background levels compared to traditional Western blotting were examined.

METHOD

50µg total protein lysate from mouse liver and lung were resolved on two 4-20% SDS polyacrylamide gels and transferred to PVDF membranes using *Efficient*™ Western Transfer Buffer (Cat. No. 82021-236).

SWIFT™ Western Diluent Procedure

Following transfer, the blot was rinsed with deionized water to remove residual transfer buffer. 10ml undiluted Swift™ Western Diluent was added on to the blot and placed on a shaker for 30 seconds to ensure both sides of the blot were uniformly coated. A mouse primary antibody against actin was then directly added to the blot in Swift™ Western Diluent at a 1:5,000 dilution. The PVDF membrane was incubated with shaking for 30 minutes at room temperature.

After incubation, the blot was removed from the tray and excess antibody solution was removed by shaking. The blot was transferred to a fresh, clean and dry tray and a 1:10,000 HRP conjugated goat anti-mouse antibody (Cat. No. 82022-742), diluted in Swift™ Western Diluent, was added and incubated for 30 minutes with shaking.

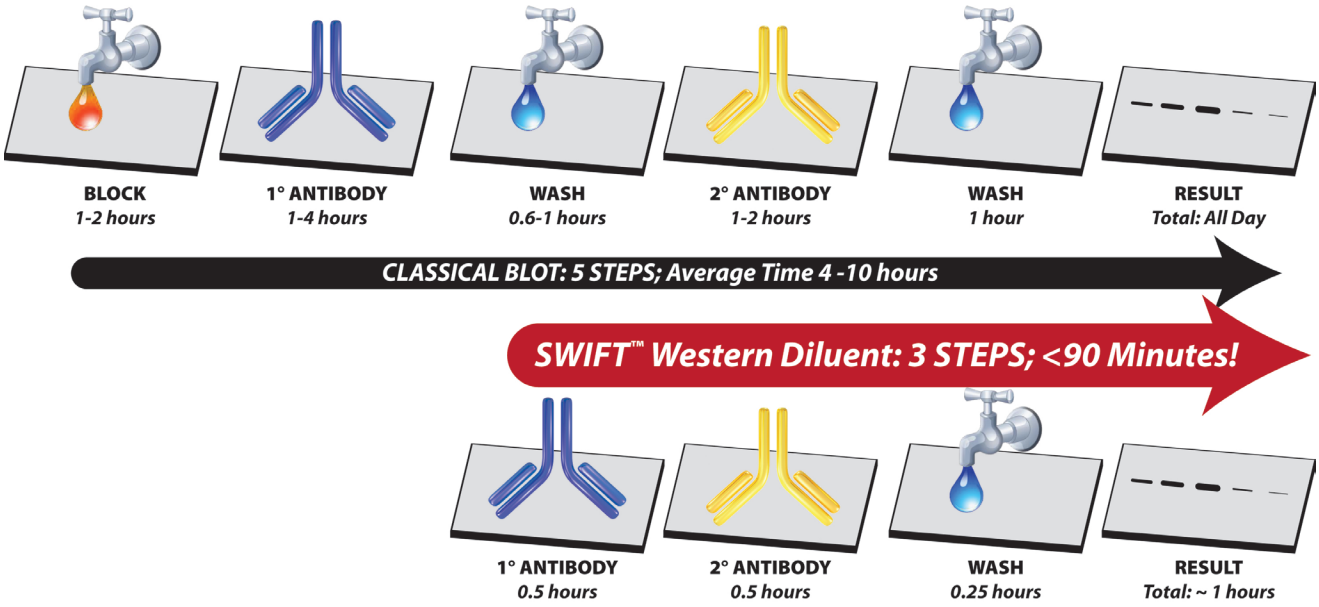

Figure 1: Traditional Western blotting compared to SWIFT™ Western Diluent. Top: Traditional Western blotting involves five key steps before development, including a minimum one to two hour block. Bottom: SWIFT™ Western Diluent is essentially a three step process; the long blocking step is eliminated.

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BIOSCIENCES®

VWR™

Traditional Western Blot Procedure

Following transfer, the blot was rinsed with deionized water to remove residual transfer buffer. Ten milliliters of NAP-Blocker™ was added to the blot and incubated for 90 minutes at room temperature. After blocking, a mouse primary antibody against actin (diluted 1:5,000 in NAP-Blocker™) was added to the blot and incubated for 30 minutes at room temperature with shaking.

The blot was washed 3 times (for 5 minutes each time) in PBS and was then transferred to a fresh, clean and dry tray. 1:10,000 HRP conjugated goat anti-mouse antibody (Cat. No. 82022-742), diluted in NAP-Blocker™, was added to the blot and incubated for 30 minutes with shaking.

After secondary antibody incubation, both blots were removed from the trays and excess solution was removed by shaking. The blot was then transferred to a fresh, clean and dry tray and washed 3 times, 5 minutes each, in 25ml wash buffer. After washing, the blot was developed using G-Biosciences' femtoLUCENT™ PLUS (Cat No. 82022-458), according to the kit protocol.

RESULTS AND DISCUSSION

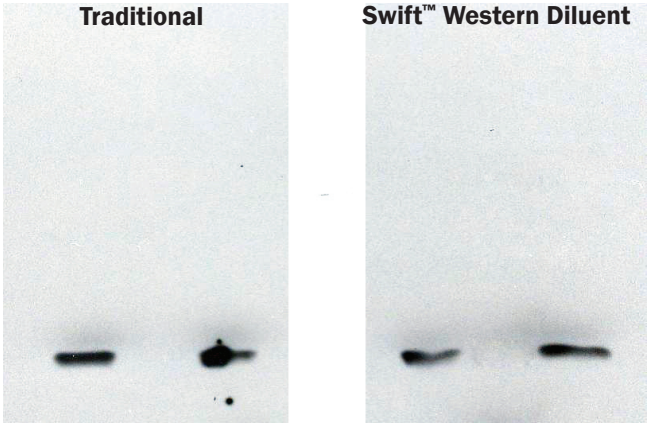

Figure 2: Traditional Western blotting compared to SWIFT™ Western Diluent. Left: Traditional Western blotting method showing the actin protein in liver and lung lysates. Right: SWIFT™ Western Diluent was used to eliminate the blocking step and developed comparable actin protein bands and a clean background.

The results show that both methods resulted in clear actin bands with clear background. When compared with the traditional method, Swift™ Western Diluent did not exhibit any detrimental effects on primary and secondary antibody treatments or chemiluminescence detection steps. The Swift™ Western Diluent method does not require a blocking step and saves time without loss of sensitivity or increased background intensity.

ORDERING INFORMATION

VWR Cat. No.	Description	Size
71003-234	SWIFT™ Western Diluent	125 mL
82021-236	Efficient™ Western Transfer Buffer [20X]	1 L
82022-626	NAP (Non Animal Protein)-BLOCKER™ [2X]	2 x 500 mL
82022-458	femtoLUCENT™ PLUS HRP *No antibodies*	1500 cm^2
82022-742	HRP labeled goat α-mouse IgG	2 mL