automated western blot development with the western processor system

Introduction

Western blotting as a technique for detecting and identifying proteins is commonly used in life science research today. A compilation of literature citation frequencies from the Medline database and first publication dates shows the evolution of the technique over time (Figure 1). The technique has changed very little, except for the type and sensitivity of the detection method used. Western blotting is a time-consuming technique that typically requires 3–5 hours for most protocols.

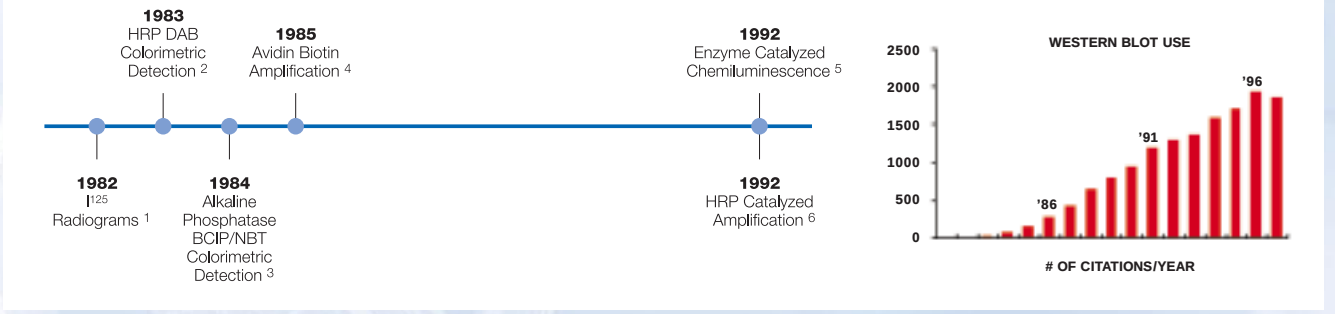


Figure 1. *Evolution of western blotting techniques over time.*

The evolution of western blot detection from I^{125} secondaries to multistep methods logically calls for the process to be automated. The need for a method that is fast and sensitive, and does not require film and a darkroom, or extensive labor, is evident from the methods chosen over the years. Where high sensitivity is not an issue, simple cost-effective colorimetric detection methods have found wide use, yielding quick results on the blot. Amplified colorimetric assays with higher sensitivity have not been as widely accepted due to the many extra manipulations required. Enzyme catalyzed chemiluminescent detection has been very popular because its sensitivity rivals isotopic detection. However, this method still requires costly x-ray film and darkroom space. A new method, peroxidase catalyzed amplification for colorimetric detection, has sensitivity similar to chemiluminescence without the x-ray film and darkroom, but requires additional blot manipulations.

While western blot processing steps vary with the detection system employed, all methods include multiple steps that can be tedious and time-consuming for the researcher. These steps require frequent hands-on time (changing buffers and adding reagents), leaving little time between steps for other work. The Western Processor system from Bio-Rad allows walk-away processing of western blots by automating the liquid handling steps required in all blot processing protocols. The Western Processor system was used to process identical blots with several detection systems in order to evaluate the quality of the instrument's output.

automated western blot development

Materials and Methods

Human transferrin (Sigma T-3309) was serially diluted and run in a 12% Tris-HCl Ready Gel (Bio-Rad) in the Ready Gel Electrophoresis Cell (Bio-Rad) for 35 minutes at 200 V constant voltage. Electrophoretic transfer to nitrocellulose membrane (Bio-Rad) was completed with the Mini Trans-Blot® cell (Bio-Rad) for 35 minutes at 200 V constant voltage. Rabbit anti-human transferrin primary antibody (Dako A0061) diluted 1:3,000 was used in each detection assay. Detection systems used for processing the blots were the Immun-Blot® alkaline phosphatase goat anti-rabbit assay kit (Bio-Rad), Opti-4CN™ goat anti-rabbit detection kit (Bio-Rad) and the Amplified Alkaline Phosphatase Goat Anti-Rabbit Immun-Blot assay kit (Bio-Rad). All assays required 20 minutes of set up time which is added to the total processing times.

Colorimetric Blot Processing With Alkaline Phosphatase and BCIP/NBT Substrate

The Western Processor system's pre-programmed "IBLOT" protocol (Table 1) was used to process the blot with the Immun-Blot alkaline phosphatase goat anti-rabbit assay kit. The protocol includes nine steps and is completed in 4.25 hours. The results from this assay are shown in Figure 2a.

Colorimetric Blot Processing With Horseradish Peroxidase and Opti-4CN Substrate

The Western Processor system's pre-programmed "OP4CN" protocol (Table 2) was used to process the blot with the Opti-4CN goat anti-rabbit detection kit. The protocol includes nine steps and is completed in 4.33 hours. The results from this assay are shown in Figure 2b.

Amplified Western Blotting With Alkaline Phosphatase and BCIP/NBT

The Western Processor system's pre-programmed "AMPAP" protocol (Table 3) was used to process the blot with the Amplified Alkaline Phosphatase Goat Anti-Rabbit Immun-Blot assay kit. The protocol includes eleven steps and is completed in 5.6 hours. The results from this assay are shown in Figure 2c.

Table 1: IBLOT Protocol

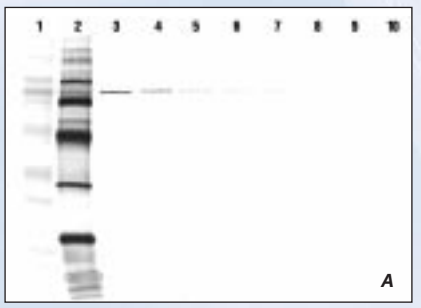
STEP	TOTAL TIME	HANDS-ON
1: Wash	5 min	
2: Block	60 min	
3: Wash	15 min	
4: Primary Antibody	60 min	
5: Wash	15 min	
6: Secondary Conjugate	60 min	
7: Wash	20 min	
8: Color Development	10 min	10 min
9: Water	10 min	
Total Time (hours)	4.25 hr	0.5 hr

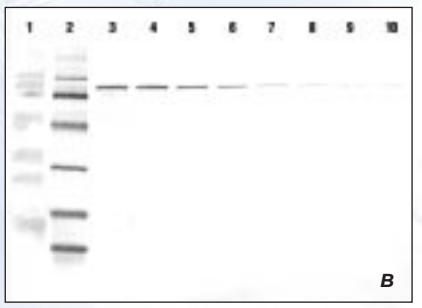
Table 2: OP4CN Protocol

STEP	TOTAL TIME	HANDS-ON
1: Wash	10 min	
2: Block	60 min	
3: Wash	15 min	
4: Primary Antibody	60 min	
5: Wash	15 min	
6: Secondary Conjugate	60 min	
7: Wash	20 min	
8: Color Development	10 min	10 min
9: Water	10 min	
Total Time (hours)	4.33 hr	0.5 hr

Table 3: AMPAP Protocol

STEP	TOTAL TIME	HANDS-ON
1: Wash	10 min	
2: Block	60 min	
3: Wash	15 min	
4: Primary Antibody	60 min	
5: Wash	15 min	
6: Secondary Conjugate	60 min	
7: Wash	15 min	
8: Streptavidin-AP	60 min	5 min
9: Water	20 min	
10. Substrate	10 min	10 min
11. Wash	10 min	
Total Time (hours)	5.6 hr	0.6 hr





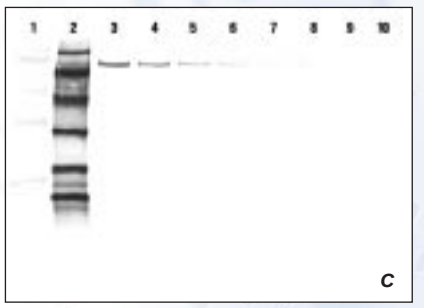


Figure 2
a. *Immun-Blot alkaline phosphatase goat anti-rabbit assay kit*
b. *Opti-4CN goat anti-rabbit detection kit*
c. *Amplified Alkaline Phosphatase Goat Anti-Rabbit Immun-Blot assay kit*

Each blot was allowed only 10 minutes for color development. Sensitivity can be enhanced with longer substrate development. Lane 1: low molecular weight prestained SDS-PAGE standards; Lane 2: low molecular weight biotinylated standards; Lanes 3-10: human transferrin: 25 ng, 12.5 ng, 6.25 ng, 3.125 ng, 1.56 ng, 0.78 ng, 0.39 ng, 0.20 ng

Conclusions

All blots processed with the Western Processor system yielded good sensitivity and low background. The Western Processor System reduced hands-on time for blot processing in all three assays tested. The most significant time savings (~5 hours) was achieved with the amplified detection system.

The Western Processor system can process one large blot or two mini blots simultaneously using the same protocol (although the capacity can be doubled by incubating two blots back-to-back in each dish if some background staining is acceptable). This system comes with five pre-programmed protocols for use with Bio-Rad kits as well as the ability to store ten user-defined protocols with up to six different reagents and fifteen steps. In addition, the system can be used as a rocker for simple gel staining applications.

References

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