APPLICATION NOTE

Speed to Results: One-Minute Western Blots with SignaLOCK™ HRP ChemiWestern Kit (Imager)

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The popularity of imaging systems is growing among researchers performing Western blot assays. These systems offer several advantages over image acquisition with film. Imaging systems provide a wider dynamic range than film[1], multiple images can be obtained, and the images are easily manipulated with software. Despite the advantages that imagers provide, some researchers have avoided these systems as a result of the prolonged exposure time needed with luminol-based chemiluminescent substrates and a prepared milk blocker in order to achieve the equivalent sensitivity of film.

To reduce the exposure time required with an imager, KPL has developed a new Western blot kit capable of high-sensitivity detection within a short period of time. The combination of the kit components, the acridan-based chemiluminescent substrate, LumiGLO Ultra™, the non-protein polymer blocker, SignaLOCK Blocking Solution, and 20X Wash Solution Concentrate, generate quality images at picogram levels of detection with a 1-minute exposure with a Peltier-cooled CCD camera enhanced imager[2]. This Application Note demonstrates that lower protein concentrations are visible after 1 minute using KPL's SignaLOCK HRP ChemiWestern Kit (Imager) than after 30 minutes with other common chemiluminescent systems. This high-sensitivity kit combined with advanced digital technology can significantly shorten exposure time on an imager.

DIRECT ASSAY DETECTION PROTOCOL

1. 3-fold serial dilutions of 1 µg/10 µL purified Human IgG were separated via SDS-PAGE, transferred to nitrocellulose, and blocked overnight at 4ºC in one of the following blocking solutions: Milk Diluent/Blocking Concentrate Kit (KPL Catalog No. 50-82-01), 5X SignaLOCK Blocking Solution (KPL Catalog No. 50-58-00), SuperBlock (Thermo Scientific), or Prime Block (GE Healthcare). Each blocking solution concentrate was diluted per manufacturer's instructions prior to blocking.

2. Western blots were washed 3 times for 5 minutes with 20X Wash Solution Concentrate (Catalog No. 50-63-00), diluted 1:20 in reagent quality water.

3. Horseradish Peroxidase (HRP)-labeled Goat Anti-Human IgG (H+L) (Catalog No. 074-1006) was diluted 1:300,000 in the corresponding blocking solution and incubated for 30 minutes at room temperature on a platform shaker.

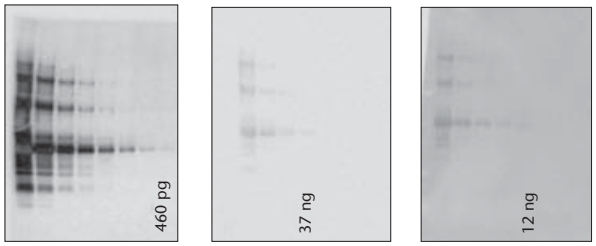
4. Western blots were washed once for 15 minutes, followed by 3 times for 5 minutes each in diluted 20X Wash Solution Concentrate (see Step 2), and once for 5 minutes in reagent quality water.

5. LumiGLO Ultra Chemiluminescent Substrate (Catalog No. 54-51-00), SuperSignal West Dura (Thermo Scientific), and ECL Prime (GE Healthcare) were prepared and used according to manufacturer's directions.

6. Western blots were placed in the G:BOX Chemi XR5 Imager (Syngene) and exposed for 1 and 30 minutes.

SignaLOCK Blocking Solution *Milk/Diluent Block*

Figure 1. While LumiGLO Ultra is effective at producing a signal within 1 minute using a milk blocker, the same Western blot blocked with SignaLOCK Blocking Solution produces noticeably stronger and clearer signal.



LumiGLO Ultra/ SignaLOCK Block *ECL Prime/ Prime Block* *SuperSignal West Dura/SuperBlock*

Figure 2. KPL's SignaLOCK HRP ChemiWestern Kit (Imager) produces more clear, bright protein bands within 1 minute when compared to other combinations of substrates and blockers at 1 minute.

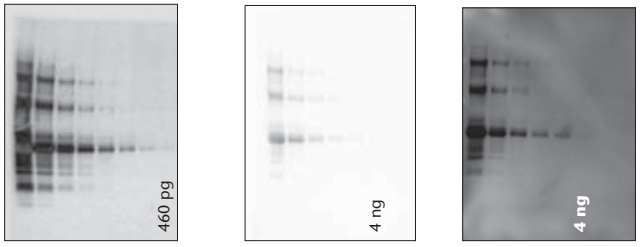


Figure 3. KPL's SignaLOCK HRP ChemiWestern Kit (Imager) produces clear, bright protein bands within 1 minute and outperforms the other combinations of substrates and blockers at 30 minutes.

RESULTS

In comparing blocking solutions, SignaLOCK Blocking Solution enabled detection of 10 nanograms, whereas 300 nanograms were visible using the milk-based blocker (Figure 1). Combining the recommended substrates and blocking solutions with a 1-minute exposure in the imager produced the following results: SignaLOCK HRP ChemiWestern Kit (Imager) detected 460 picograms, ECL Prime Blocking Agent/ECL Prime detected 37 nanograms, and SuperBlock/ SuperSignal West Dura detected 12 nanograms (Figure 2). When the SignaLOCK HRP ChemiWestern Kit (Imager) blots were exposed for 1 minute, 460 picograms were visible, whereas when ECL Prime Blocking Agent/ECL Prime and SuperBlock West Dura/SuperSignal were exposed for 30 minutes the result was 4 nanograms each (Figure 3). This demonstrates that additional imager time could not overcome the performance advantages of high sensitivity and low background achieved by the SignaLOCK HRP ChemiWestern Kit (Imager).

PRODUCTS

Description	***Size***	***Catalog No.***
SignaLOCK HRP ChemiWestern Kit (Imager)	1000 cm^2	54-54-00
SignaLOCK AP ChemiWestern Kit (Film/Imager)	1000 cm^2	54-56-00
5X SignaLOCK Blocking Solution	125 mL	50-58-00
LumiGLO Ultra Chemiluminescent Substrate	50 mL	54-51-00
HRP-labeled Goat Anti-Human IgG (H+L)	1.0 mg	074-1806
Milk Diluent/Blocking Concentrate Kit	1.0 mg	50-82-01

SUMMARY

Researchers are always looking for tools to improve the efficiency of their immunoassays. Despite the fact that analysis with advanced imaging systems is preferred due to the wider dynamic range and ease of storing images relative to film, the amount of exposure time required with luminol-based substrates and milk blockers is known to be inconvenient. Unfortunately, comparable exposure time with film using traditional substrates and blockers results in grainy images with dim protein bands, reinforcing the notion that superior Western blot results requires long exposure times on imagers. This study demonstrated that by switching to a high-sensitivity detection kit, image acquisition times can be reduced to 1 minute on an imager. For picogram limits of detection, the combination of reagents provided in the SignaLOCK HRP ChemiWestern Kit (Imager) produces blots with a stronger signal and lower background with a 1-minute exposure in an imager when compared to other systems after 30 minutes.

REFERENCES

1. Martin, C. S., Bronstein, M.l. Imaging of Chemiluminescent Signals with Cooled CCD Camera Systems. J Biolumin Chemilumin. 1994; 9:145 – 153.

2. Sluder, Greenfield, Wolf, David E. eds. Methods in Cell Biology. San Diego (CA): Academic Press; 1998. Chapter 3, Cooled CCD Versus Intensified Cameras for Low-Light Video – Applications and Relative Advantages; p. 45 – 62.

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