Chemiluminescence Detection of Transferrin at Picogram Dynamic Range

Introduction

Within the field of protein analysis, researchers are demanding imaging systems to capture low abundance proteins in a dynamic range down to picogram level. The most sensitive but costly detection method is to use direct fluorescence detection as opposed to the popular and trusted chemiluminescence detection method. However, with the advanced Peltier cooled charged coupled device (CCD) camera and high-quality chemiluminescence detection reagents widely available today, capturing low noise-to-signal chemiluminescent images at picogram dynamic range is a cost-effective and reliable option. KETA C systems the dedicated chemiluminescence imaging systems are the ideal choices for researchers capturing low abundance proteins on chemiluminescent blots at picogram dynamic range with low background.

Materials and Methods

Protein detection

Detecting protein samples on chemiluminescent blots was performed with purified human transferrin (≥95% by SDS-PAGE, BioVision). A two-fold dilution series of transferrin from 312.5 picogram to 1.2 picogram was made. The Bis-Tris gel casting and gel electrophoreses were performed with V-GES Vertical Gel Electrophoresis System (V-GES, Wealtec). The gel electrophoreses (4% and 10% SDS-PAGE) were run for 40 minutes at 60 V and then for 70 minutes at 120 V. The wet transfers were run for 200 mA for 1 hour using the PVDF membranes (Immobilon®-P, Merck) and the E-blotter module of the V-GES system. The membranes were blocked with 5% skim milk for 1 hour, incubated in 1:15 000 transferrin antibody overnight (15 hours), and then incubated in 1:50 000 HRP-(horse radish peroxide) conjugated antibody for 1 hour. The antibody conjugated proteins were

detected using the chemiluminescence detection reagent Chemi-Lumi One Super (nacalai).

Image capture and analysis

The membranes were captured using the Auto Capture Mode to determine the best exposure time before they were saturated with KETA CX Chemiluminescence Imaging System (KETA CX, Wealtec) and Magic Chemi Image Capture and Analysis Software (Magic Chemi, Wealtec). Images were analyzed with Spot Analysis in Magic Chemi to determine the limit of detection and dynamic range. The Spot Standard function was used to plot mass vs. int OD to see the linearity of protein samples. To check the noise-to-signal level, Gel Analysis was used to demonstrate the noise-to-signal ratio.

Results

Picogram dynamic range

Transferrin was conjugated with primary antibody and then with HRP-conjugated secondary antibody to be detected via chemiluminescence reagent. Detection limit and dynamic range for transferrin are presented in Figure 1-3. Transferrin conjugated with HRP-conjugated antibody was detected down to 1.2 pg on KETA CX. The Spot Analysis in Magic Chemi was used to determine the mass based on the gray level detected by the CCD camera. The graph was plotted int OD (Gray Level) versus mass in the Spot Standard function (Figure 2) to show the best fitted line for reference. The same graph was plotted again on Excel (Figure 3) to show the linearity $R^2 = 0.9905$.
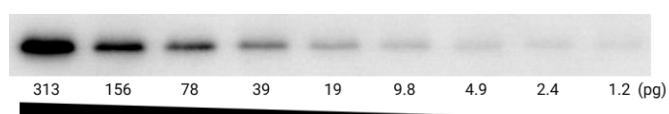

Figure 1 Two-fold dilutions from 313 to 1.2 pg transferrin detected via Chemi-Lumi One with KETA CX and Magic Chemi.
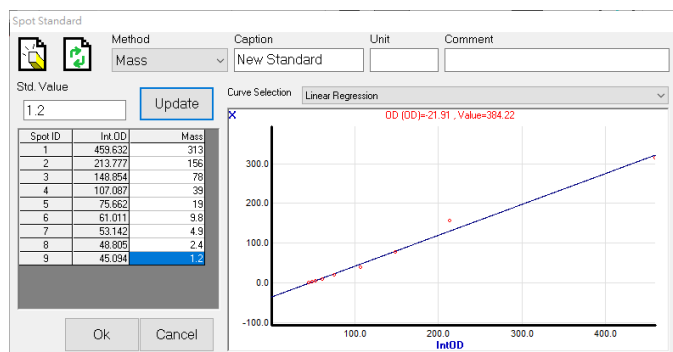
Figure 2 Int OD (Gray Level) versus mass (pg) of the protein chemiluminescent signals plotted from Figure 1 using the Spot Standard function in Magic Chemi. Each known concentration (two-fold dilution from 313 to 1.2 pg) was assigned each band to show the linearity on the best fitted line.
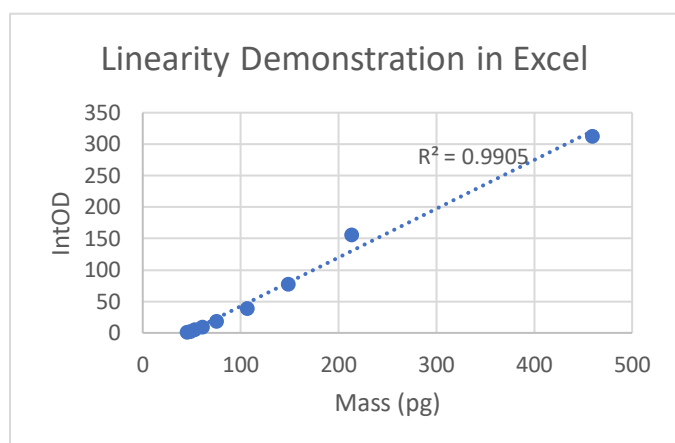

Figure 3 The best fitted line was plotted from the table in the Spot Standard function in Magic Chemi shown in Figure 2 to demonstrate the linearity R² = 0.9905

Noise-to-signal image demonstration

The noise-to-signal images were demonstrated with Lane Profile in Gel Analysis. Each band in the two-fold dilution series from 313 to 1.2 pg was taken to demonstrate the noise presented in the background along with the signal, or the highest peak.
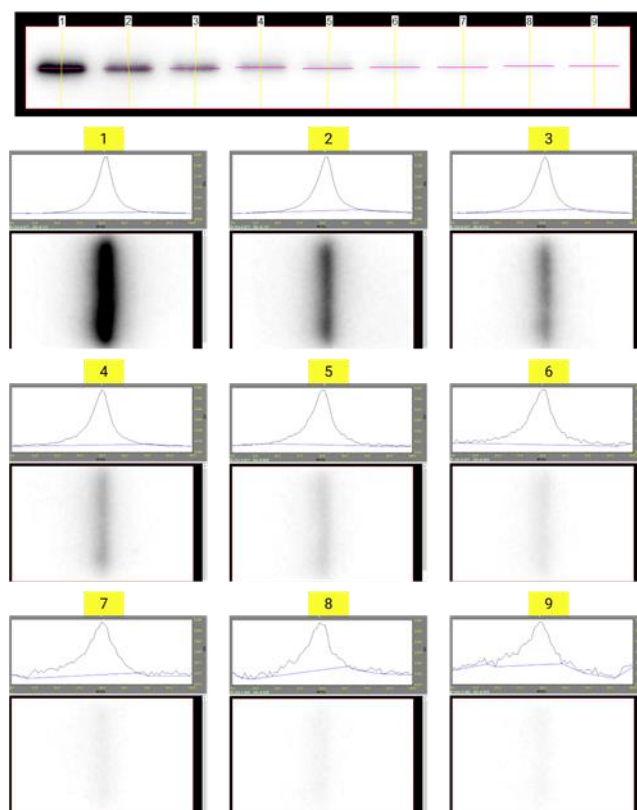

Figure 4 Noise-to-signal demonstration of the HRP-conjugated transferrin blots using Lane Profile in Gel Analysis in Magic Chemi.

Discussion

While there are several factors affecting the noise level of chemiluminescent blots, KETA CX is capable of detecting proteins at picogram dynamic range with optimized blots. Transferrin amino acid composition and antibody dilution affect how transferrin conjugates with antibodies and how background noise interrupts the chemiluminescent signals. Thus, small variation in detection limits can be expected. In testing the limit of detection, the purified transferrin protein was not denatured with reducing agent and heat to avoid disrupting the purified protein structure. To ensure capturing low noise-to-signal blots, optimizing the blots with different transfer conditions and different antibody dilutions may be required. The sensitivity of an imaging system is determined by the ability to capture low signal-to-noise ratio images. The CCD cameras in KETA C Chemiluminescence Imaging Systems are capable to achieve low noise image

background and thus high sensitivity of the blots.

Conclusion

Capturing low abundance proteins on chemilumi-nescent blots at picogram dynamic range is best recommended with KETA C the dedicated chemilu-minescence imaging systems. As long as the blots are optimized with the antibody dilution and sam-ple preparation, the low noise-to-signal back-ground can be achieved through the Peltier cooled CCD cameras of the KETA C systems.

Ordering Information

Catalog no.	KETA C Imaging Systems
1150065	KETA C
1150075	KETA CX
1150085	KETA CLX

Catalog no.	KETA C Software and Accessories
1153005	Magic Chemi software package for KETA C series, single user key only
1153006	Magic Chemi software package for KETA C series, three user keys
1153007	Magic Chemi GLP/GMP software, single user key only