Chemiluminescence Signal Detection by Peltier Cooled CCD

INTRODUCTION

When captured gel images in imaging systems, non-cooling CCD camera equipped with 8 or 12 bits of the pixel depth was largely used as the common equipment. Sensitivity of all these imaging systems is much enough for the general gel image capturing. As Wealtec provides the cooling CCD detector into the general gel documentation systems for KETA GL and KETA GLX, it increases not only the S/N ratio in capturing images but also gives the detection ability for normal gel imaging system to observe chemiluminescence samples. Procedures that illustrated in this article will show how to do the best chemiluminescent detection in general gel documentation.

MATERIALS

- KETA GL imaging system (Wealtec)
- Human Lung cancer cell (a549) total cell lysate
- Anti-GAPDH antibody, anti-mouse secondary antibody (Santa Cruz)
- Human Transferrin (Sigma)
- Anti-Transferrin antibody, goat anti-rabbit secondary antibody (Epitomics)
- Immobilon™ Western Chemiluminescent HRP Substrate (Millipore)

PROCEDURES

- Western blot assay

1. Diluted samples were separated in 12% SDS-PAGE with V-GES and ELITE series power supply.
2. After electrophoresis, protein samples were transferred from gel onto blotting membrane by using Wealtec's transfer system under a proper blotting condition (Yrdimes for a549 cell lysate and E-blotter for transferrin protein, respectively).
3. Membranes were blocked by BSA solution at the room temperature for an hour, and then hybridized with primary antibody for one more hour and then with secondary

antibody for the other one hour. After hybridization, membranes should be washed for 10 minutes with TBST buffer for 3 times.

For total cell lysate:

Primary Ab: anti-GAPDH antibody; Secondary Ab: goat anti-mouse antibody

For purified human transferrin protein:

Primary Ab: anti-Transferrin antibody; Secondary Ab: goat anti-rabbit antibody

4. Chemiluminescent signals were excited by adding ECL substrate and detected by KETA GL imaging system.

- Antibody spot test

1. Nitrocellulose (NC) membranes were pretreated with TBST buffer and then air-dried for 10 minutes.
2. Diluted Goat-anti-mouse secondary antibodies were blotted onto the pretreated NC membrane with volume of 1 µl.
3. After further 10 minutes air-dried, membranes were added with ECL substrate directly to evoke the chemiluminescent signals.
4. Chemiluminescent signals were detected by KETA GL imaging system.

RESULT

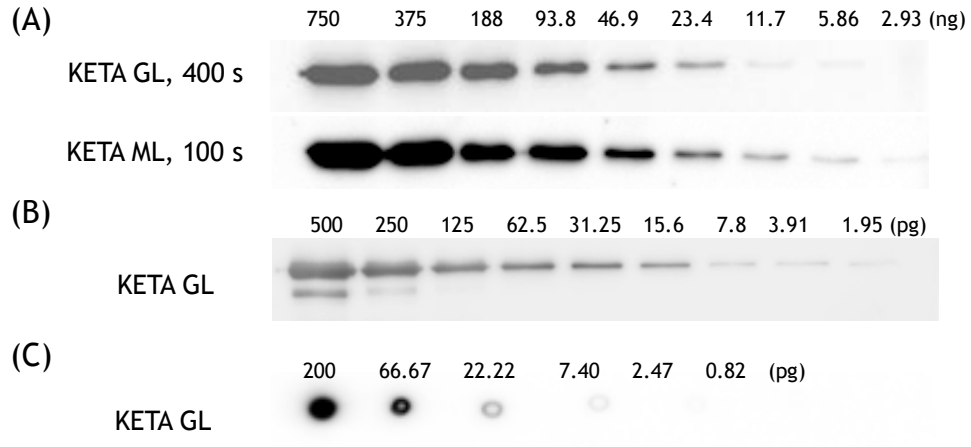

Figure 1. *Chemiluminescent Detection by KETA GL imaging system.*

(A) ***GAPDH*** *in total cell lysate in KETA GL and KETA ML. (B)* ***Pure transferrin*** *with 60 seconds exposure. (C)* ***HRP conjugatged goat-anti-mouse antibody*** *with 80 seconds exposure.*

DISCUSSION

Using of peltier cooled CCD in KETA GL and KETA GLX imaging systems is assumed having the ability of detecting the chemiluminescent samples. Detection ability had also been proved in this article (*fig*.1). Since KETA G series are not designed specifically for chemiluminescence observation, the ability of detection for the same sample was lower than multifunction imaging system, which is the KETA ML. As in *fig*.1 (A), with the same sample detection, it took 4 times longer to capture the signal in KETA GL system. The detection limit was also one level lower than KETA ML system. Besides, the signal intensities of each band in KETA GL were all lower than those in KETA ML.

KETA GL system equipped with peltier cooling system was proved to have the ability to capture the chemiluminescence signal, but not as easy as in KETA ML system. Following conditions should be noticed to have best detection result in detecting chemiluminescence sample with KETA GL:

1. Sample tray should be raised up by using 5~10 cm height box to have the sample much closer to the camera to increase the capturing possibility.
2. Make sure that there is no filter in front of the lens.
3. Enlarge the iris setting to maximum.
4. Make sure to have correct focal adjustment with the door opened.
5. Extend the exposure time to have more than 100 seconds when start capturing under the preview mode in the capturing interface.
6. Turn on the binning function to have 2x2 or 4x4 setting to increase the signal intensity.

Detecting chemiluminescent signal by high performance gel documentation, KETA GL is an alternative way to satisfy general chemiluminescence applications. Comparing with research grade KETA ML chemiluminescence system, KETA GL is a much cost/performance effective system than others.

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