Detection Limitation of KETA ML Imaging System

INTRODUCTION

Since the KETA ML has the most sensitivity second stage Peltier-cooled CCD, when applying with very low concentration chemiluminescence samples, users can get the lowest detection demand amount of samples. As there are too many different samples used in these kinds of experiments, it is not easy to define the sensitivity of the imaging system. Many factors will also affect the detections, such as the sample conditions with cell lysate or purified samples, the intensity of protein-antibody reaction, and even the interaction intensity between the 1st and the 2nd antibody. Even with the different capture setting of the image system, the detection limit will be different. In this article, the sensitivity of KETA ML imaging system was defined in two typical conditions for users' references.

EQUIPMENTS AND MATERIALS

- KETA ML imaging system (Wealtec)
- V-GES system (Wealtec)
- Blotting systems: E-Blotter (Wealtec)
- Human MCF7 cell lysate and purified GST protein was obtained from Graduate Institute of Physiology in National Taiwan University College of Medicine.
- ß-Actin - 1st antibody: anti-ß-Actin (Sigma), 2nd antibody: anti-mouse-IgG-HRP.
- GST protein- 1st antibody: mouse-anti-GST, 2nd antibody: anti-mouse-IgG-HRP.
- Chemiluminescent: ECL Enhanced Chemiluminescence reagent (Millipore)

PROCEDURES

1. Western blot experiment was performed by the laboratory in Graduate Institute of Physiology in National Taiwan University College of Medicine.

2. Serial dilutions of human cell lysate were separated with 12% SDS-PAGE with followed samples: (1) Total protein with amount of 78, 57, 36, 27.5, 19, 9, 4.5, and 2.25 ng. (2) Purified GST protein with amount of 75, 38, 19, 9.4, 4.7, 2.35 and 1.17 pg.
3. After electrophoresis, transfer the protein onto the PVDF membrane.
4. Hybridization with the 1st and then the 2nd antibody.
5. The result was presented with ECL Enhanced Chemiluminescence reagent.
6. Detect the result through KETA ML image system by using batch capture and DynaView method with no binning setting.

RESULTS

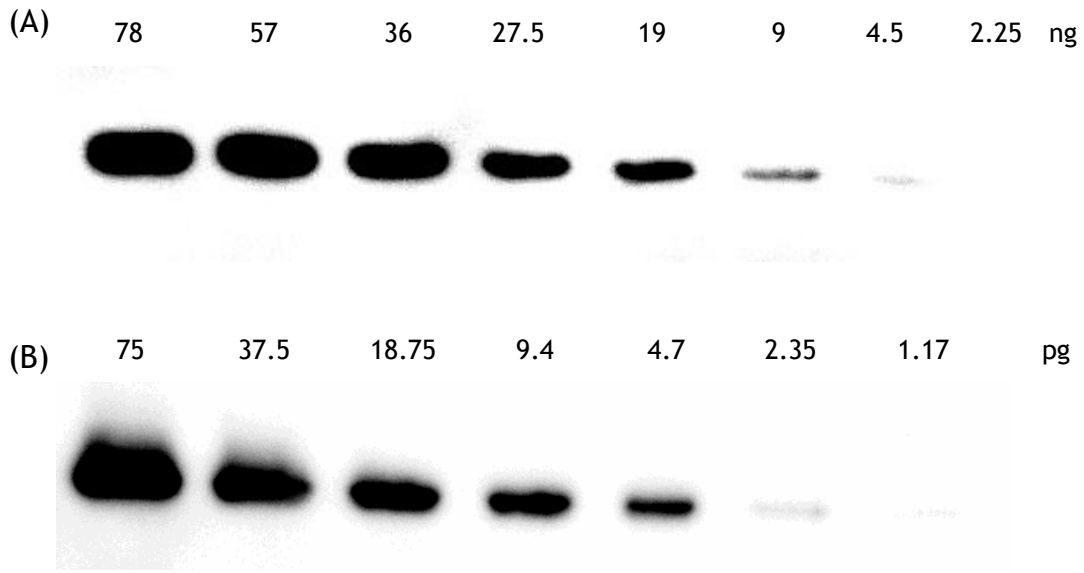

Figure 1. *Chemiluminescence detection via KETA ML imaging system. (a) Detect of β-actin in cell lysate with 20 seconds DynaView exposure for 8 pictures. (b) Detect of Pure GST protein with 20 seconds DynaView exposure for 2 pictures.*

DISSCUSION

Conditions of samples in chemiluminescence experiment play an important role on defining the detection limitation of the image systems. As in *fig*. 1, detection limitation with ß-actin in total cell lysate can be defined at 4.5 ng, but can be defined at 2.35 pg with purified GST protein. The detection level of both two samples has over than 1,000 fold differences in the protein amount. It is all because of the different sample conditions. Beta-actin is a housekeeping protein which can be easily be found in many different cell lines with less than 1% to the total cell proteins. When using of 4.5 ng total cell lysate as sample, the actual ß-actin content will be around 45 pg or even lower. So, when calculate the amount of the ß-actin in total cell lysate, the detection limitation should be also defined as ”pico-gram” level as well.

On the other hand, using of different antibodies will also affect the determination of sensitivity in image system. As example in *fig*. 1, detecting ß-actin with anti-ß-actin antibody and GST protein with anti-GST antibody can result in 45 pg and 2.35 pg respectively. All the pictures in this article was captured under the condition of un-activated binning function and could be a reference for users to determine the performance of KETA ML system, but it is not the absolute value for KETA ML sensitivity. Users can always improve the detection ability of KETA ML imaging system as they want by applying with the binning function, tuning the gain value of camera, and extending the exposure time. All these enhancing functions are available in Wealtec powerful molecular image system, KETA ML imaging system.

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