Chemiluminescence Detection with Keta ML Imaging System

INTRODUCTION

Equipped with the highest level 2-stage Peltier cooled CCD, Keta ML imaging system is the best option among Keta M series for user to do the Chemiluminescence experiment. Forty five degree Celsius below ambient is that K12CHS can reach largely lower down the noise of the CCD camera. While detecting the chemiluminescence, there have a lot of luminal reagent on the market for user to choose. Among those various detection intensity reagents, stronger light emission reagent can get faster exposure but also easier to make the high concentration signal saturated or decayed. Though the weaker one cannot be detected easily but it can be observed with much lower sample amount. According to above, using of correct luminal reagent is very important in chemiluminescence detection.

EQUIPMENTS AND MATERIALS

- Human MCF7 cell lysate obtain from Graduate Institute of Physiology in National Taiwan University College of Medicine.
- 1st antibody: anti-ß-Actin (Sigma), 2nd antibody: anti-mouse-IgG-HRP.
- ECL Enhanced Chemiluminescence reagent (Millipore)
- Western Lightning Plus-ECL (Perkin Elmer)
- Keta ML imaging system (Wealtec)

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 the total

protein amount of 156, 117, 78, 57, 36, 27.5, 19, and 9 ng.

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 and Western Lightning Plus-ECL.
6. Detect the result through Keta ML image system by using batch capture method.

RESULTS

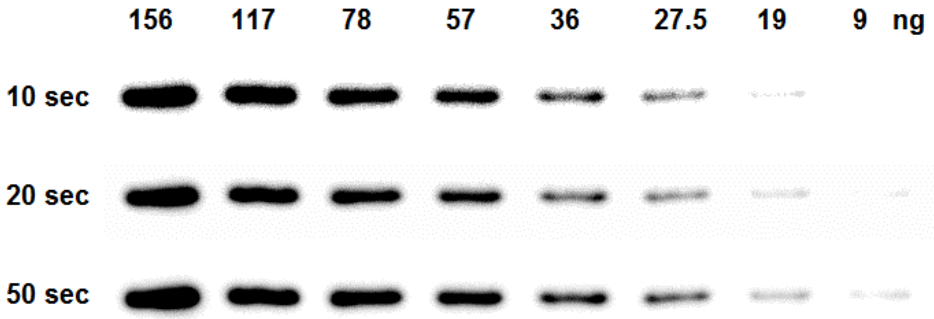

Figure 1. *Using of **Plus-ECL** to detect β-actin in series diluted human cell lysate via Keta ML image system with 10, 20, and 50 seconds exposure.*

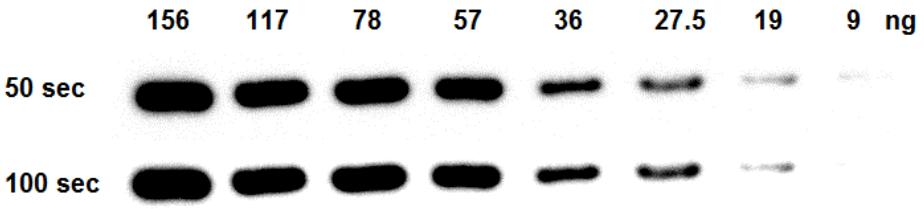

Figure 2. *Using of **ECL Enhanced** to detect β-actin in series diluted human cell lysate via Keta ML image system with 50 and 100 seconds exposure.*

DISSCUSION

While detecting lower concentration of chemiluminescence target samples, using of correct luminal reagent can be the critical point that makes the experiment success. As in the figure 1, using of ECL Enhanced luminal reagent and observe with Keta ML image system, it takes only few seconds to capture the image. Along with the increasing exposure time, the minimum sample amount that can be detected is around 9 ng of the total protein within 20 seconds exposure. In the same sample condition but using of different luminal reagent (*Fig*. 2), as long as the exposure time increase, the absorbance of high amount sample will easily get over-exposed while the lowest amount is not detected yet. Also, some of the reagents are not available for longer exposure time but have decayed bands after expose for period of time. Both above situations will lower down the detection sensitivity of the samples. It's not cause of the lower quality camera, but due to the wrong luminal reagents.

With the comparison of the using different ECL luminal reagents, Keta ML system was proved again to have very high sensitivity (9 ng total protein) and good image quality on detecting chemiluminescent signals. With using of proper luminal reagent and prolonging the exposure time, Keta ML imaging system can have much higher sensitivity.

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