Intensify the Signal Detection of KETA ML Imaging System

INTRODUCTION

KETA ML imaging system equipped with the highest S/N ratio camera among whole KETA series imaging system was tested to have detection limit with 9 ng of total protein in the previous bulletin. Using of the same transfer condition but extend the exposure time with lower sample amount, 2-stage Peltier cooled CCD was tested to have better detection limit in the Plus-ECL presented experiment.

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.
- 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 78, 57, 36, 27.5, 19, 9, 4.5, and 2.25 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 Western Lightning Plus-ECL.
6. Detect the result through KETA ML imaging system by using DynaView method in Magic Chemi software with 20 seconds exposure time each and 10 pictures accumulation.

RESULTS

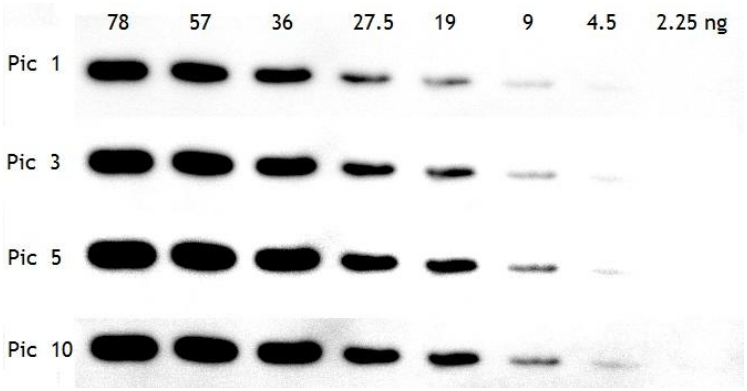

Figure 1. *Using of **Plus-ECL** to detect β-actin in series diluted human cell lysate via KETA ML image system with DynaView method. Pic1~Pic10: DynaView setting with 10 pics and 20 seconds each. Numbers are the amount of total protein.*

DISSCUSION

During detecting of the low concentration chemiluminescence samples, there have few ways to intensify the signal intensity; for example, expending the exposure time, accumulate the signal, and lower down the noise signal. All these methods are designed in the Magic Chemi software to enhance the chemiluminescence signal detection in KETA imaging system. In figure 1 is the typical application of detecting chemiluminescence signal. Expending the exposure time to 20 seconds and accumulate the signal by using DynaView category with 10 pictures accumulation, the detection limit of KETA ML imaging system can be reached at 4.5 ng total protein which is lower than the previous test. Operator is suggested to apply these built-in methods to intensify their weak signal image detection.

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