Chemiluminescent Detection of GAPDH, Pure Transferrin, and Mono-clone Antibody via 2-Stage Cooled CCD

INTRODUCTION

The method for determining the sensitivity of CCD camera would be varied according to the detection samples, ranging from protein in total cell lysate and pure protein Western blot, and mono-clone antibody-HRP dotting detection. Each detecting methods toward the samples would be used to determine the sensitivity of the camera in very different way. Some methods can detect with nano-gram level of the samples but others pico-gram. Here have some typical sample applications for second stage Peltier cooled CCD camera in KETA ML imaging system for users to evaluate what KETA ML can do.

EQUIPMENTS AND MATERIALS

- KETA ML imaging system, V-GES, E-Blotter, and Yrdimes (Wealtec)
- Lung cancer a549 cell lysate
- GAPDH - 1st antibody: anti-GAPDH, 2nd antibody: goat-anti-mouse-IgG-HRP. (Santa Cruz).
- Transferrin (Sigma)- 1st antibody: Rabbit-anti-Transferrin, 2nd antibody: HRP conjugated goat-anti-rabbit antibody (Epitomics).
- PVDF and NC membrane (Millipore)
- Chemiluminescent: ECL Enhanced Chemiluminescence reagent (Millipore)

PROCEDURES

Western blot of GAPDH and pure transferrin:

1. Run the 12% SDS-PAGE with series diluted cell lysate and pure transferrin solution as followed amount: (1) Total cell lysate with amount of 750, 375, 187.5, 93.75, 46.88, 23.44, 11.72, 5.86, and 2.93 ng. (2) Purified transferrin protein with amount of 125, 62.5, 31.25, 15.63, 7.81, 3.9 and 1.95 pg.

2. Transfer the total lysate from SDS-PAGE onto PVDF membranes by using Yrdimes for 25 V, 10 minutes, and pure transferrin protein by using E-Blotter for 100 V, 1 hour.
3. Hybridization the target protein with relative antibody, respectively.
4. Present with ECL and take pictures with KETA ML imaging system with different exposure time without turning on the binning function.

Detection of HRP conjugated antibody:

1. Treat the NC membranes with TBST for 20 seconds.
2. Dry the membranes for 10 minutes.
3. Dilute the HRP conjugated Goat-anti-mouse antibody with following concentrations: 200, 66.67, 22.22, 7.40, 2.47 and 0.82 pg/μL.
4. Spot the samples on the membranes with 1 μL.
5. Dry the membrane for another 10 minutes.
6. Add with ECL Enhanced Chemiluminescence reagent.
7. Observe the result with KETA ML imaging system with different exposure time without turning on the binning function.

RESULTS

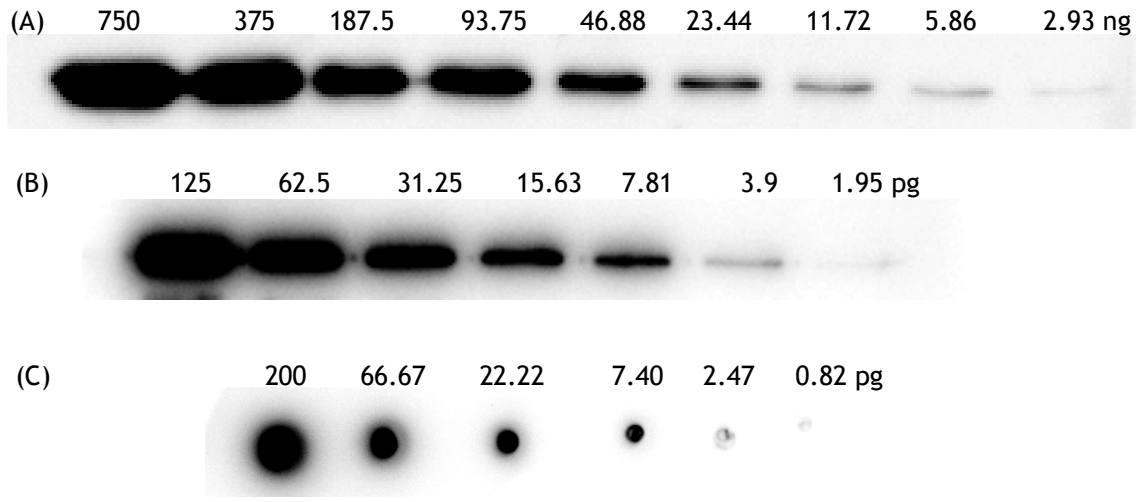

Figure 1. *Detection of chemiluminescent samples in KETA ML imaging system. (A)* ***GAPDH*** *in total cell lysate with 100 seconds exposure. (B)* ***Pure transferrin*** *with 10 seconds exposure. (C)* ***HRP conjugatged gaot-anti-mouse antibody*** *with 80 seconds exposure.*

DISSCUSION

When talking about the detection sensitivity of a camera, customers would like to know how low of the sample amount can be detected. Because of all the samples are too varied, no one could define the sensitivity in a specific sample concentration. It only can be defined as a reference detection level for sort of sample types, for example pico-gram or nano-gram level for pure protein or total protein. As detecting with common targets in total cell lysate (*fig*.1 (A)), the detection limitation of KETA ML is around 2.93 ng by detecting GAPDH. For pure protein detection, the detection limit can be lower down to detect the 1.95 pg pure transferrin protein (*fig*.1 (B)). Compare to the previous experiment in technical bulletin #25, the detection level of KETA ML can be defined around 2~3 ng level for the reference protein in total lysate, and around 2~3 pg level for pure protein. Since the detection limitation will affected by many factors during operation, mostly the transfer efficiency, detect with dotting methods can largely lower down the undesirable effects. In *fig*. 1(C), dotting with HRP conjugated antibody can easily be performed and the detection limitation of KETA ML is about 0.82 pg after 80 seconds exposure.

Detecting the chemiluminescent samples with cooled CCD, the cooling ability of CCD plays an important role in detection limitation. Using of second stage Peltier cooled CCD can provide user the best observation with lowest noise signal. About detection of the real samples, KETA ML imaging system was defined with very good performance in this article for users' references.

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