Superior Chemiluminescent Detection with High S/N Ratio 2-Stage Cooled CCD Camera

INTRODUCTION

As one of the most different specifications in the KETA C series is the cooling ability of camera, KETA C equipped with one stage Peltier cooling system K12CH camera and KETA CL with two stage Peltier cooling system K12CHS camera. As having the same 2/3" size but better cooling ability on top of the CCD chip, KETA CL can have higher signal to noise ratio. But, whether better S/N ratio can guarantee to have better detection limit or not, there is no prove of it. In order to evaluate the relationship between cooling ability and performance, CCD cameras were performed in two systems by using the same lens to eliminate all other affecting factors. Detection limit of two imaging systems were also defined after adjust the capture condition for several times.

MATERIALS

- KETA C and KETA CL imaging system with f0.95 lens (Wealtec)
- Goat anti-mouse-IgG conjugated with HRP (Santa Cruz)
- Immobilon™ Western Chemiluminescent HRP Substrate (Millipore)
- Nitrocellulose membrane (Millipore)

PROCEDURES

1. Before performed the experiment, KETA C series systems were performed with over than 2 hours darkroom calibration and were also adjusted to have best observation conditions.
2. Prepare the serial diluted goat anti-mouse-IgG antibody into 1x PBS buffer with following concentrations:
   a. 400, 80, 16, 3.2, 0.64 pg/μL
   b. 400, 100, 25, 6.25, 1.56, 0.39 pg/μL
   c. 400, 100, 50, 6.25, 0.78, 0.09 pg/μL

3. Immerse the NC membrane with PBS buffer for five minutes
4. Put the membrane on filter paper to dry for another five minutes.
5. After the membrane dried out, dot the series diluted antibody solutions with 1 μL onto NC membrane.
6. Wait for at least five minutes for those dots to dry out
7. Present with mixed ECL reagent and remove unnecessary solution.
8. Put the membrane into KETA C or CL imaging systems and place on the top layer of tri-level stages.
9. Detect with DynaView function with the setting of capturing for 10 times and 10 seconds exposure time for each capturing.
10. Adjust the sample amount and repeat the image capturing with different setting to check the image with the best conditions.

RESULT

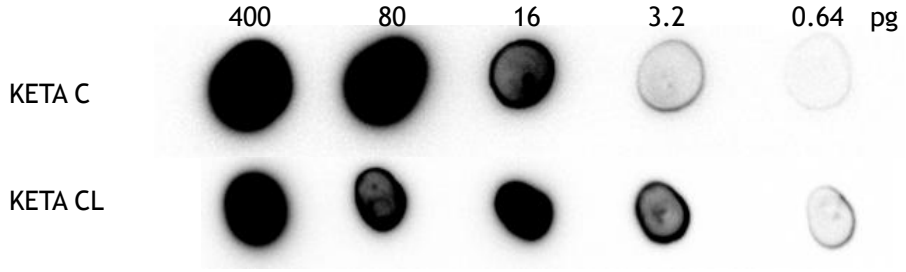

Figure 1. *Series diluted antibody dots captured with KETA C and CL imaging systems through DynaView function. Setting with capturing for 8 pictures and exposuring 10 seconds for each.*

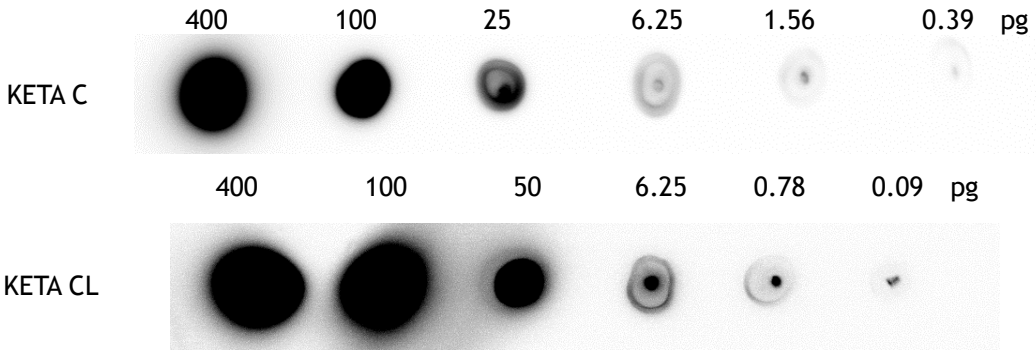

Figure 2. *Detection limits of KETA C and CL imaging systems. Captured with 10 pictures with exposure time for each with 10 seconds in KETA C and 10 pictures with exposure time for each with 20 seconds in KETA CL system in DynaView function.*

DISCUSSION

While comparing capturing ability of two cameras, all settings, including lens usage, capturing mode, aperture setting, and sample distance should be fixed with exactly the same conditions. Under these same capturing conditions with total 80 seconds exposure time, both one and two stages cooled 2/3" CCD camera can have similar observation result with 0.64 pg of the antibody dot. While comparing with results in previous articles about KETA ML imaging system with K12CHS camera, KETA C with K12CH camera can have better detection due to be designed especially to focus on chemiluminescence detection. Comparing among the same KETA C series systems, stronger signal detection was found on the 0.64 pg antibody spot in two stages cooled K12CHS CCD camera than one stage K12CH CCD camera.

As lower down the sample amounts for detection and adjust the best condition for detection to find the detection limit of both cameras, KETA C can get very amazing result with 0.39 pg detection after adjust the image contrast and brightness. About the second stage cooled CCD, KETA CL can have 0.09 pg detection limit with total 200 seconds exposure detection which is much sensitive than in KETA C systems. In conclusion, there is no question about two stage cooled CCD can having better sensitivity or detection limit than one stage CCD camera.

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