Sensitivity Improvement in Chemiluminescence Detection

INTRODUCTION

Since photon illuminate energy from chemiluminescence sample is easily decayed as travel through distance, while detecting samples, target should be put as closer as possible. Also due to the degradation of the photon energy, detection limit of one imaging system will also define differently in different object distance. In KETA C series system, it equipped with tri-level sample stage that can detect the optimize signal for different sample size. Moreover, since completed with Magic Chemi, it also provided with various enhancing functions including Binning, Batch Capture, and DynaView functions for chemiluminescent sample detection. Some functions had been described in previous article. Now, as the chemiluminescence-oriented system, sensitivity results were showed in different level and by using the binning function in the KETA CL system.

MATERIALS

- Goat-anti-mouse-IgG-HRP (Santa Cruz)
- Immobilon Western Chemiluminescence HRP substrate (Millipore)
- NC membrane (Millipore)
- KETA CL imaging system (Wealtec)

PROCEDURES

1. Goat-anti-mouse-IgG-HRP antibody was diluted to proper concentration with PBS.

Spot	1	2	3	4	5	6
Protein amount(pg/ul)	10	5	2.5	1.25	0.67	0.34

2. NC membranes were moistened with PBS buffer and then dried briefly.
3. Drop 1 µl series diluted antibody onto NC membrane and then dried briefly.
4. Membranes were added with ECL reagent.
5. Image was taken by KETA CL imaging system in different level stages.
6. Capturing modes setting:

Capturing mode	DynaView	DynaView	DynaView
Binging function	off	2x2	4x4
Repeat Exposure Number	10	10	10
Exposure Time (sec)	30	30	30

RESULT

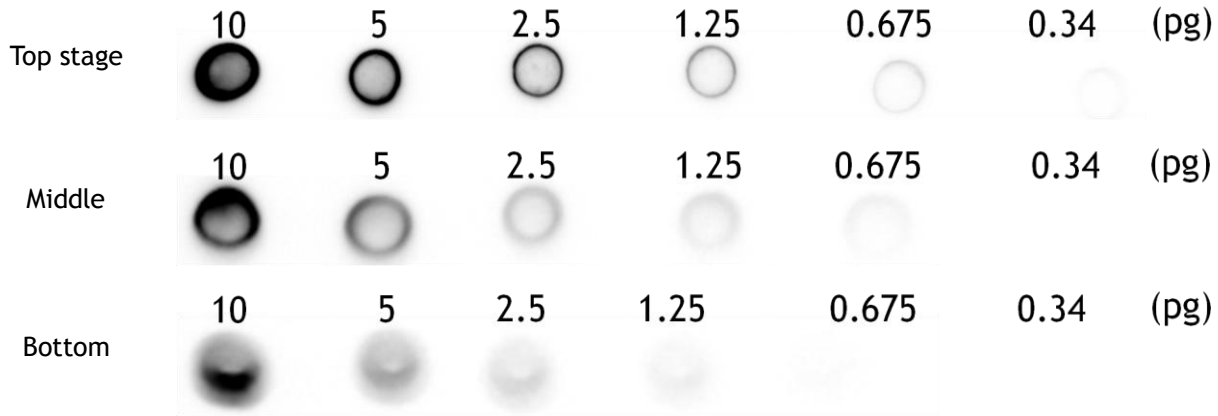

Figure 1. *Sample detection on different level stages with 300 seconds exposure.*

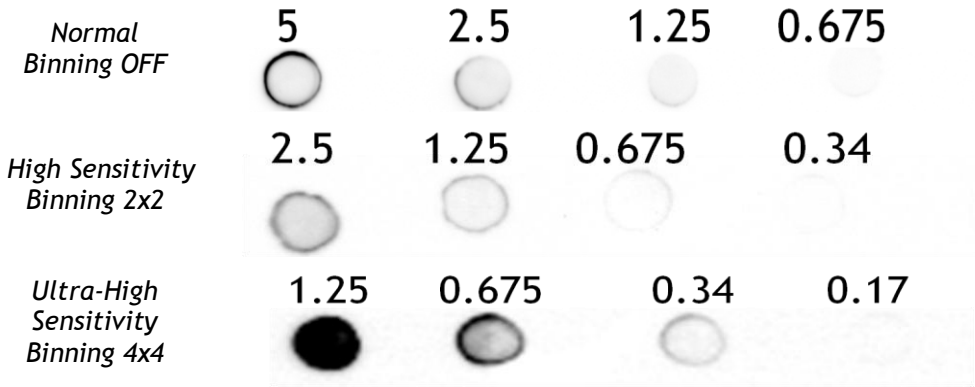

Figure 2. *Sample detected with different binning function on top stage.*

DISCUSSION

All data that been presented here were all performed by using antibody sample dots and captured image without any modifications, because western blot result is influenced by many factors, such as purity of protein samples, transfer efficiency, quality of antibody and detection reagents, and even imaging systems setting.

Compared the detection sensitivity on different stages (as figure 1), users can get the best detection ability of KETA CL when put the sample on the top stage. As the object distance to the camera increases, the detection ability gets weaker. The detection sensitivity is 2 fold different by each stage. Besides, as enhancing the detection ability through Magic Chemi software by activating the Binning function, result as showed in figure 2. Without activated the binning function, the detection sensitivity is about 0.675 pg. As setting with high sensitivity (binning 2x2) function, detection ability was improved with 2 folds lower in sample concentration. Whereas captured with ultra-high sensitivity (4x4 binning) function, detection sensitivity was largely enhanced with 4 folds lower in sample concentration and even better.

Regarding the different detection sensitivity, the observation area of top stage is approximately 6 cm X 5 cm area, 16 cm X 12 cm for middle stage, and 24 cm X 18 cm for bottom stage. The tri-level stage provides a wide range of observation area for users to choose their optimal stages to get the best sensitivity. Besides, three strategies of binning functions provide the other way to improve the signal detection.

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