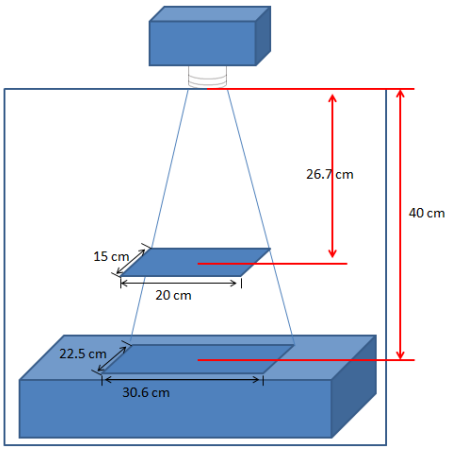
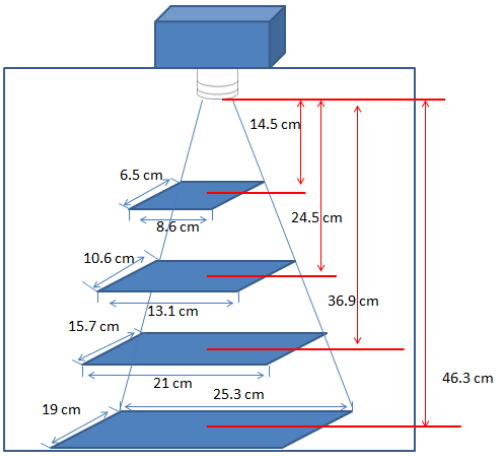
Compare Efficiency between KETA CL and KETA ML in Chemiluminescent Detection

INTRODUCTION

Both systems equipped with two-stage Peltier cooling system K16C II camera (ambient -25°C) which ideal for ultra-high sensitivity chemiluminescent detection. Both have better cooling ability of the CCD camera to provide higher signal to noise ratio in chemiluminescent signal detection. Designed base on different application purposes, object distance of these two imaging systems were varied as followed table:

Table 1. *KETA ML versus KETA CL*

Model	**KETA ML**	**KETA CL**
Lens	F1.4 motorized lens	F1.4 prime Lens
Object Distance And Effective Area		
Applications	Gel Documentation, Fluorescent, and Chemiluminescent Detection	Dedicated system for Chemiluminescent Detection

As KETA CL is designed specific targeting on chemiluminescent detection, object distance is varied according to the object size. But in KETA ML system, as need to satisfy all applications, sample can only be placed with fixed object distance. Among these differences, the detection result of two systems is varied. Through detecting with the same target sample with same protocols, minutes different can be detected as in following result.

MATERIALS

- BCA protein assay kit (Novagen)
- Primary antibody: anti-ß-Actin (Novus Biologicals) and anti-GAPDH antibody (abcam)
- Secondary antibody: anti-mouse-IgG-HRP (Santa Cruz)
- Western Lightning ECL Pro (Perkin Elmer)
- 0.45 µm pore size PVDF membrane (Perkin Elmer)
- Electrophoresis systems: V-GES system (Wealtec)
- Blotting systems: E-Blotter (Wealtec)
- KETA CL and KETA ML imaging system (Wealtec)
- SpectroArt 200 (Wealtec)

PROCEDURES

1. Determine the total protein concentration by using BCA methods and measured through SpectroArt 200 spectrophotometer.
2. Prepare the serial dilutions of human A549 cell lysate with following amount per 10 µl: 1880, 940, 470, 235, 117.5 and 58.75 ng.
3. Separated the protein sample in 12% SDS-PAGE at 100 volt for 2 hr.
4. After electrophoresis, proteins were transferred to PVDF membranes at 100 volt for 1 hr. 5% non-fat milk in TBST was used as a blocking buffer.
5. The membranes were incubated with primary antibody (ß-Actin and GAPDH) under 1:5000 dilution and then with anti-mouse-IgG-HRP with 1:7500 dilution.
6. Detect the result with KETA CL and KETA ML image system. Adjust the lens setting with clear focus on the top level sample stage and with largest iris setting. Set the DynaView capture method with capture 10 pictures and 30 seconds exposure time for each capture in normal binning mode.

RESULT

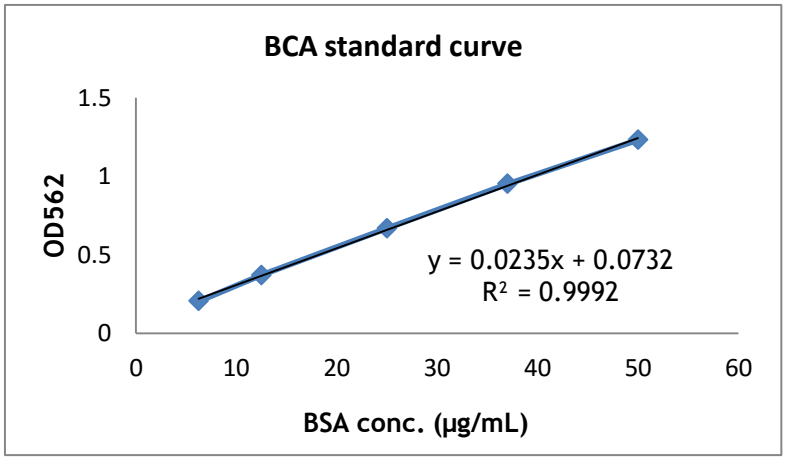


Figure 1. *BCA standard curve for protein quantification*

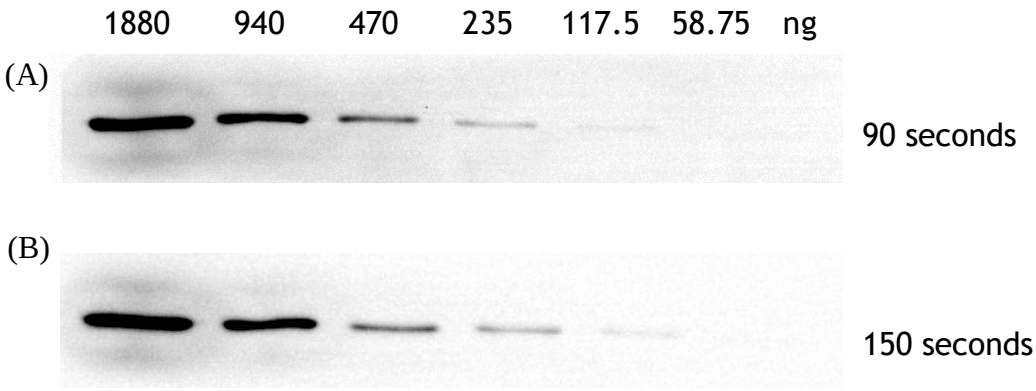


Figure 2. *Detecting **GAPDH** in series diluted human A549 cell lysate via (A) KETA CL and (B) KETA ML image system with DynaView.*

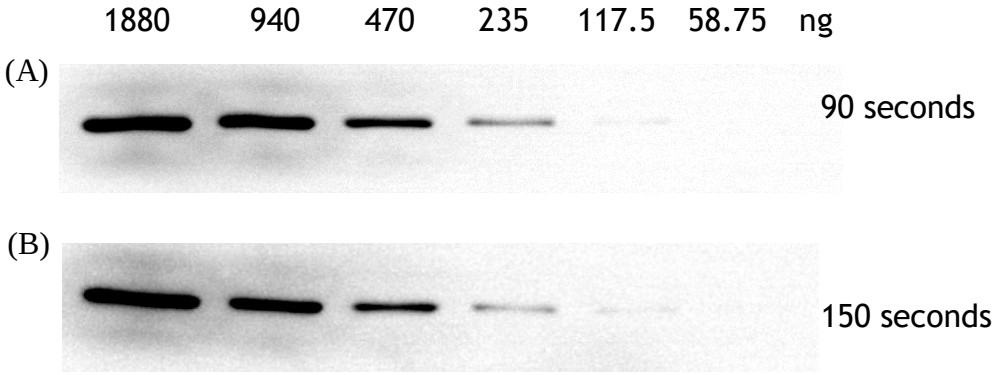


Figure 3. *Detecting **β-actin** in series diluted human A549 cell lysate via (A) KETA CL and (B) KETA ML image system with DynaView.*

DISCUSSION

As in the figure 2 and 3, signal accumulation with 90 seconds and 150 seconds through DynaView category respectively, the detection limit with GAPDH and ß-actin in total cell lysate both can be detected as low as 117.5 ng total protein. Under the similar experimental conditions, KETA CL can get better detection efficiency than KETA ML with shorter exposure time. This was resulted from the object distance difference. Chemiluminescent samples are easily decayed as travel through distance. While detecting sample, target should be placed as closer as possible toward the CCD. When detecting sample with KETA CL, sample can be placed on the top layer of the sample stage with 16.7 cm object distance, which in the KETA ML was 26.7 cm.

Besides, during detecting of the low concentration chemiluminescent samples, both systems have few ways to intensify the signal intensity; for example, extending the exposure time, accumulate the signal, applying with the binning function, tuning the parameters of camera, and lower down the noise signal. All these methods had already designed in KETA M and C series imaging systems to enhance the signal detection ability. Moreover, users can also improve the detection ability of KETA imaging system as they want applying with more sensitive reagents.

To sum up, if focusing on chemiluminescent detection only, it's highly recommended to choose KETA C series systems with lower cost, higher efficiency, and more dedicated function. Moreover, it's free from cross contamination of undesired light sources other than chemiluminescent signal. If more functions such as gel documentation, fluorescence detection, and chemiluminescent detection are required, to lose a bit efficiency but getting multi-functional unit in one model would be worth. No matter what purpose, both KETA M and KETA C series are highly sensitivity and efficiency research grade imager on the market.

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