The Performance of Dolphin-Chemi Mini

INTRODUCTION

Dolphin-Chemi Mini image system is mainly for the chemiluminescent experiment. It consists of a 16-bit CCD camera which detects the weak signal after luminal chemical development and the small dark room provides dual stage for capturing image. The design is very compact and user-friendly. Upon capturing the image, Dolphin-1D software can serve as a good analytical tool to provide further detailed information, such as band quantification. With 10 hours maximum exposure through 10 accumulations, users have the options to choose the best image form different exposure time through computer without the need to use the X-ray film.

MATERIAL AND METHOD

Cell lysate

- K562 cell line (Emo Biomedicine Corp., Taipei, Taiwan)
- NK92 cell line (Emo Biomedicine Corp.)
- Hep G2 cell line (Emo Biomedicine Corp.)

Protein electrophoresis and transfer

Load the BSA sample, K562 cell lysate, NK92 cell lysate and Hep G2 cell lysate mixed with 4X protein loading dye and a pre-stained marker into 12 % SDS-PAGE (0.75 mm) and run the electrophoresis as follows; 60 minutes at 90 V, and then 60 minutes at 130 V. After the electrophoresis, the gel was transferred on PVDF membrane by E-Blotter (Wealtec, Taipei, Taiwan). The condition used 100 V for 1 hr.

Western Blotting and ECL development

After transfer, the PVDF membrane was placed into a 5 % milk / TBST blocking solution for 30 minutes. Thereafter, the blocking solution was discarded and the membrane was incubated with a primary mouse anti-β-actin antibody (1:1000, dissolved in 5 % milk / TBST) for 1 hour in RT with agitation. The membrane washed in TBST 10 minutes x 3, and thereafter incubated with a HRP-conjugated secondary goat anti-mouse antibody (1:3000, 5 µl antibody dissolved in 15 ml 5 % milk / TBST)

for 1 hour in RT with agitation. The secondary antibody solution discarded and the membrane washed 10 minutes x 3. Therefore, 0.5 ml substrate luminal reagent and 0.5 ml substrate peroxide solution (1:1) pipetted on to the membrane and allowed to react for 1 minute.

RESULT

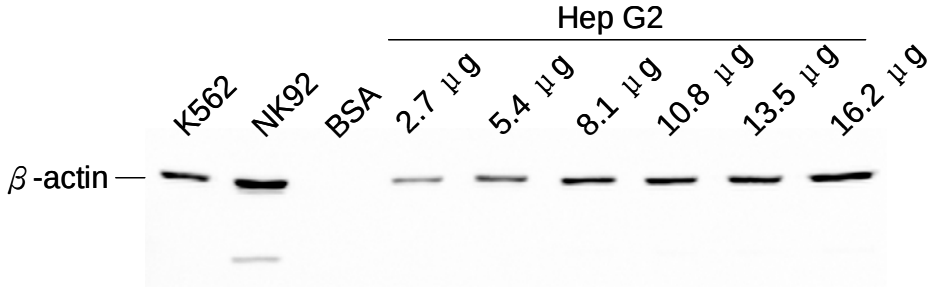

Figure 1. *β-actin series dilution in Hep G2 cell line. K562 loading 15µg and NK92 cell lysate serve as a positive control. BSA is a negative control.*

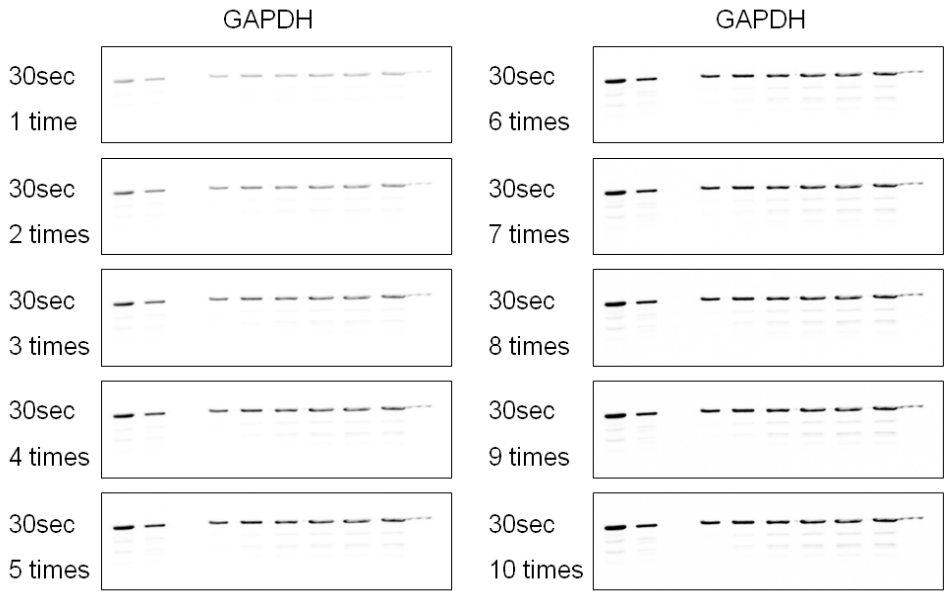

Figure 2. *Time lapse pictures of GAPDH in Hep G2 cell line. The same sample order as-per to figure 1 but incubated with different primary antibody, GAPDH.*

DISCUSSION

The total exposure time of figure 1 is 2 minutes and it was adjusted by Contrast and Histogram functions to be clearer. It shows that Dolphin-Chemi Mini can detect very low amount β-actin. However, the sensitivity of the primary antibody also plays an important role in chemiluminescent experiments. If the efficiency of the primary antibody is not good, the result of chemiluminescence would not be easy for detection.

Figure 2 shows the time lapse of GAPDH, due to the reason the β-actin images are too weak in order to see the difference between each accumulation the data is shown). These data captured with 30 sec exposure time with 10 repetition numbers integration condition. The software automatically saves each picture and allows users to choose the best images among all.

In Integration Mode, Dolphin-Chemi Mini provides the longest exposure time up to one hour with ten repetitions resulting in ten hours maximum exposure time. The image also can be adjusted and optimized with Contrast and Histogram functions without the need for long exposure time. Upon capturing images Dolphin-Chemi Mini transfers the captured image automatically to Dolphin-1D software for further analysis.

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