Performing Basic Western Blot Visualization with Dolphin-Chemi Mini

MATERIAL

- K562 cell lysate (Emo Biomedicine Corp., Taipei, Taiwan)
- NK92 cell lysate (Emo Biomedicine Corp.)
- Pre-stained marker (Bio-Rad, Hercules, CA, U.S.A.)
- 12 % SDS-PAGE resolving gel (0.75 mm); 2.31 ml ddH_2O, 2.8 ml 30 % Acrylamide/Bis (29:1) (Bio-Rad), 1.75 ml 1.5 M Tris-Cl, pH 8.8 (Sigma-Aldrich Ltd., St Louis, MO; U.S.A.), 70 µl 10 % SDS (Bio-Rad), 70 µl 10 % APS (Bio-Rad), 2.8 µl TEMED (Bio-Rad)
- 5 % SDS-PAGE stacking gel (0.75 mm); 1.7 ml ddH_2O, 0.415 ml 30 % Acrylamide/Bis (29:1) (Bio-Rad), 0.315 ml 1 M Tris-Cl, pH 6.8 (Sigma-Aldrich), 25 µl 10 % SDS (Bio-Rad), 25 µl 10 % APS (Bio-Rad), 2.5 µl TEMED (Bio-Rad)
- V-GES equipment (Wealtec, Taipei, Taiwan)
- HB-1 Block Heater (Wealtec)
- BSA (1 mg/ml), 0.05 g BSA (Sigma-Aldrich) dissolved in 50 ml ddH_2O, aliquoted in 1 ml solution per Eppendorf and freezed in -20°C.
- Mouse-anti-GAPDH antibody (Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA)
- Goat-anti-mouse IgG-HRP antibody (Santa Cruz Biotechnology, Inc.)
- TBST; 25 mM Tris, 3 mM KCl, 140 mM NaCl, 0.05 % Tween-20, pH 7.4.
- 5% milk / TBST; 2.5 g non-fat milk powder in 50 ml TBST.
- 10X Tris-Glycine buffer (Sigma)
- Immubilon™ Western – Chemiluminescent HRP substrate 100 ml (Millipore, Billerica, MA)

PROCEDURE

- A 12 % SDS-PAGE-gel was prepared. 5 ml of the resolving gel solution and thereafter 1 ml EtOH was pipetted into the assembled casting module. The gel

was polymerized for 60 minutes. After polymerization, the EtOH was removed and the stacking gel solution was prepared and poured on top of the polymerized resolving gel. A 0.75 mm 10 well comb was gently inserted into the space between the plates, ensuring no air bubbles were trapped. The stacking gel was left for polymerization for 60 minutes.

- SDS-PAGE running buffer was prepared by diluting 100 ml 10X buffer stock in 900 ml ddH_2O. To the SDS-running buffer, SDS was added to a final concentration of 3.5 mM. The gel sandwich was released from the casting module and inserted into the electrode module. A one-gel gate was inserted into the empty side of the module. When the glass plate gel sandwich was securely in place, the electrode stand lid was closed. SDS-PAGE running buffer was poured into the space between the gel sandwich and the one-gel gate up until app. 1 cm from the upper edge of the glass plates. The assembly was checked for leakage, and then 1 L SDS-PAGE running buffer was poured into the tank surrounding the gel assembly. Thereafter the comb was removed.
- The BSA sample, K562 cell lysate, NK92 cell lysate mixed with 4X protein loading dye and a pre-stained marker all were boiled for 5 minutes in 95°C on a blocker heater. 10 µl for each sample was loaded into each well.
- The lid was placed on top of the tank and the electrode cables were connected to the power supply.
- The electrophoresis was run as follows; 60 minutes at 90 V, and then 60 minutes at 130 V.
- After the electrophoresis, the gel was removed from the glass plates and the upper stacking gel was cut and discarded. The resolving gel was put into transfer buffer for equilibration. The transfer buffer was prepared by diluting 100 ml 10X buffer in 200 ml methanol and 700 ml ddH_20.
- The PVDF membrane was briefly pre-wet in methanol and then equilibrated in transfer buffer. Two filter papers and sponges were soaked in transfer buffer.
- The E-Blotter cassette was opened, and one wet sponge and a wet filter paper were placed on the black cassette. The gel was gently placed on top of the filter paper.
- A PVDF membrane cut to the same dimensions as the gel and filter papers/sponges was placed on top of the gel and any air bubbles were removed by gently stroking a spacer across the membrane. A second filter paper and then

a sponge were placed on top. The red cassette was used to close off the sandwich.

- The electrical transfer was run as follows: 60 minutes at 100 V.
- After the transfer, the PVDF membrane was recovered and subjected to further western blotting.
- 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-GAPDH antibody (1:1000, dissolved in 5 % milk / TBST) for 1 hour in RT with agitation. The membrane was 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 was discarded and the membrane was washed 10 minutes x 3.
- 0.5 ml substrate luminal reagent and 0.5 ml substrate peroxide solution (1:1) was pipetted on to the membrane and was allowed to react for 1 minute. Afterwards, the ChemiStage image system door was opened and the membrane was placed on top of the tray placed in the upper position. The door of the image system was kept open to let in ambient light to allow focus adjustments during the capture-settings.

The capture settings were performed as follows:

- The *Detailed Mode* was selected.
- The *Focus*-button was pressed to reveal a second window with the membrane displayed. An *Exposure Time* that allowed enough light in to adjust focus was chosen (e.g. 0.01 sec). The screw on the camera inside the image system securing the camera focus was loosened and the camera was manually focused. When a clear focused image of the membrane was seen in the displayed window, the screw was tightened and the door of the image system was closed.
- The *Shot Setting* was set to Integration. The *Repeat Num bar* was pulled down and five was selected.
- In the *Image Size / Shot Time*, 1360*1024 (Binning1) was chosen. Binning 1 gives a better resolution than Binning 2.
- In the *Time* pull down bar, *30 sec* was chosen.
- The *Exposure* button was clicked in order to start the image capture. After the image had been captured, the *Contrast* bar was pulled down and *Histogram*

was chosen. The contrast and brightness of the image was adjusted.

- The captured image was saved. Upon clicking the *Capture*-button in the image acquiring window, the image will be automatically transferred and displayed in Dolphin-1D for further analysis.

RESULTS

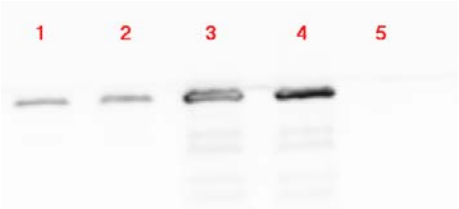

Figure 1: *Chemiluminescence data of the Western Blotting. Lane 1 and 2: K562 cell lysate; lane 3 and 4: NK92 cell lysate; Lane 5: 7.5 µg BSA solution as a negative control. The molecular weight of GAPDH is 50 KDa.*

REMARKS

- For detailed operation, other than given basic functions in this bulletin, please refer to the operation manual.
- That would be slight ECL efficiency variance between different brands. The signal might decay very fast so it is better to finish the whole procedure as soon as possible.
- If the signal is saturated, the image acquiring procedure would automatically be stopped unless the users uncheck the *Judge Over Exposure* in the *Shot Setting* at the beginning.
- The performance of the 16 bit CCD camera is very good and it is a friendly operation system whether in software or the darkroom.

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