

## Chemiluminescent western blot imaging using ChemiFast substrate

### Introduction

Western blotting is a commonly used technique to identify and quantify specific protein(s). The commonly used western blotting substrates are luminol based and produce a chemiluminescent signal. Substrates such as ChemiFast are highly sensitive enhanced chemiluminescent substrates. The ChemiFast substrate's extremely intense signal output enables detection of HRP using cooled charge couple device (CCD) camera imaging methods.

### Materials and Methods

#### Materials

G:BOX iChemi XR camera system

#### Methods

##### Sample preparation

BSA was diluted to the following concentrations loaded onto a gel 1024ng, 512ng, 256ng, 128ng, 64ng and 32ng. Samples were heated in loading buffer at 70<sup>o</sup>c for 15 minutes.

##### SDS-PAGE gel

10% separating gel and a 4% stacking gel were prepared. Gel was run at 150 volts for 1 hour 30 minutes. Once run the gel was transferred onto a PVDF membrane (Santa-Cruz Biotechnology, USA) at 300amps for 1 hour.

##### Blocking

PVDF membrane was incubated for 2 hours in 5% BSA solution diluted in PBST.

##### Antibody incubations

Blot was incubated overnight at 4<sup>o</sup>c with primary antibody BSA mouse monoclonal (Santa-Cruz Biotechnology, USA). After washing blot with PBST the membrane was incubated with secondary antibody HRP- conjugated goat anti-mouse (Santa-Cruz Biotechnology, USA).

##### Detection method

ChemiFast working solution was prepared by mixing equal parts of stable Peroxide solution and the luminol/Enhancer solution. A total of 5mls was prepared.

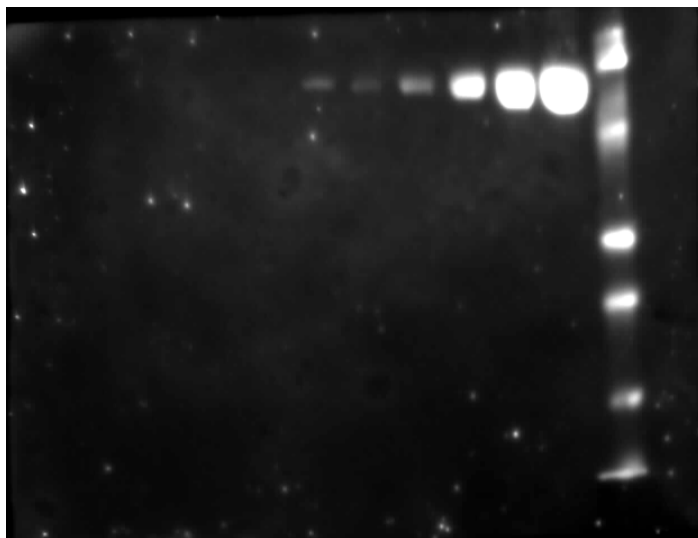
The blot was incubated with the working solution for 5 minutes at room temperature in the dark.

##### Visualization

The blot was placed in a plastic membrane protector, then placed onto a black viewing platform inside the CCD imaging system.

The following lighting and filter combination were used No lighting and no filter.

### Results



**Figure 1- Image of western blot using ChemiFast substrate.**

Image was captured using a G:BOX iChemi XR system and iChemi software. The image was captured for an exposure time of 1m29s. From right to left the following concentrations of BSA were loaded onto the gel 1024ng, 512ng, 256ng, 128ng, 64ng and 32ng.

### Conclusions

ChemiFast substrate provides 24 hour light emission and in combination with Syngene's CCD camera imaging systems multiple exposures can be performed for publication quality blots.

The substrate's intense signal and enhanced sensitivity ensures faint bands are detected.

***Syngene reserves the right to amend or change specifications without prior notice. This Application Note supersedes all earlier versions.***

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