peqlab a VWR company

FUSION™ Multi-Imaging Systems

Western blotting: Sensitivity, resolution and quantification superior to any X-ray film

Chemiluminescence detection...

...by Western blotting has become standard practice for the analysis of particular proteins in e.g. whole cell lysates. With its high specificity and sensitivity this technique is used in nearly every molecular biology and biochemistry lab. Thereby scientist are able to rapidly and reliably detect even smallest amounts of protein within the femtogram range.

The technical principle...

…of chemiluminescence detection is based upon the catalysis of Luminol, a cyclic diacylhydrazide that is oxidized in the presence of H_2O_2 by a peroxidase (such as horseradish peroxidase, HRP) thereby emitting light. After electrophoretic separation (typically PAGE) proteins are immobilised onto an appropriate membrane through 'blotting' and hybridised for instance with HRP-coupled antibodies. Nonspecific bound antibodies are washed away, before the membranes are incubated with a Luminol containing ready-to-use substrate solution like AceGlow™ from PEQLAB. The emitted light is then detected via X-ray film or digital imaging systems to verify the presence of specific antigens.

The limits of X-ray films...

...become obvious as soon as signal detection is to be quantified. Since with the application of the X-ray film darkening of light sensitive particles (usually silver halide molecules) is a 'all-or-nothing' reaction, no linearity between intensity of the emitted signal and darkening of for example protein bands can be determined. Especially after digitalisation by an image scanner, grayscale depth and resolution are further decreased. Images generated in this way are therefore not suitable for quantification. Consequently, many scientific journals now decline publications of densitometric data gathered from X-ray film.

'The FUSION™ Multi-Imaging systems with their high sensitivity, extremely low background and great versatility have defined the top rank in their class. Offering a near endless number of chemiluminescence, bioluminescence and fluorescence imaging applications the FUSION™ family meets even the highest experimental demands. Our goal is to provide you with the most of data integrity and fidelity through unmodified and uncompromised raw images. To draw substantiated scientific conclusions any post-processing, image enhancement or modification is absolutely under the control of the operating scientist. Furthermore, with the included software sophisticated data analysis options like quantification and 3D dynamic scan allow to retrieve a maximum of information depth from your experiments. Highest technical standards meet scientific precision to guarantee the successful completion of your project.'

Dr. Marcello Stein, Product Manager

Digital imaging systems...

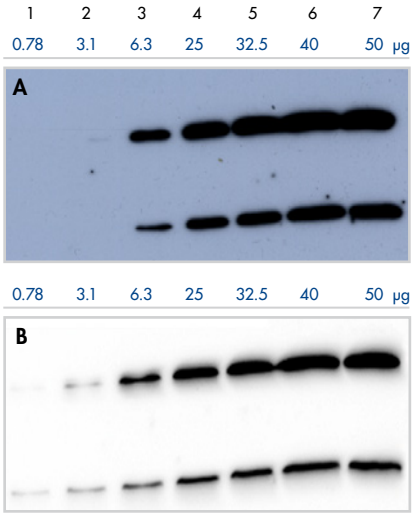
...generate real quantifiable and therefore publishable data, as the sample's emitted light is immediately detected by the CCD array and converted into electrical signals with a direct linear correlation between their amplitude and signal intensity. For best detection and results the FUSION FX7™ Advance, FUSION SL™ Advance and FUSION Solo™ imaging systems use a highly sensitive 4.2/10.0 megapixel CCD sensor with a 16 bit greyscale depth that translates into 65,536 different light intensities.

Experimental comparison...

...of both detection methods highlights the advantages of digital imaging: 0.78 µg to 50 µg whole cell lysate were electrophoretically separated by SDS-PAGE, transferred onto a nitro cellulose membrane and subsequently stained with HRP-conjugated antibodies. Figure 1 shows the comparison between Western blot imaging via conventional X-ray film (A) and the FUSION FX7™ digital imaging system (B).

Results clearly indicate...

…that the FUSION FX7™'s high sensitivity allows detection of far smaller protein concentrations when compared to conventional X-ray film. After 5 minutes of exposition 0.78 µg of protein were clearly visible with the image generated by the FUSION FX7™, whereas the X-ray film showed no signal until 6.3 µg (Fig. 1). Especially signal separation and clarity of the bands are far superior with the FUSION FX7™. Additionally, the densitometric analysis with the Bio-1D software suite confirmed that sensitivity, dynamic range and, most importantly, linearity between applied protein amount and signal intensity are best achieved with the FUSION FX7™ system. The X-ray film's missing greyscale depth makes it impossible to faithfully quantify the applied protein amount (Fig. 2), whereas the FUSION FX7™ system allows reliable quantification.



Actual customer data. Used antibodies and molecular weight omitted for confidentiality reasons.

Fig. 1. Western blot analysis of ascending amounts of protein from whole cell lysate (0.78 µg – 50 µg). Exposition time 5 minutes.
(A) Scanned X-ray film.
(B) Image generated with the FUSION FX7™ digital imaging system.

	1	2	3	4	5	6	7
A	-	-	19.7	35.8	45.6	51.0	50.0
B	0.9	3.4	10.3	29.2	36.1	44.0	50.0

Fig. 2. Densitometric analysis of Western blot signals with the BIO-1D software suite. Both signals in each lane were quantified and normalized to 50 µg (lane 7). Numbers represent the quantified protein amounts in µg as detected with conventional X-ray film (A) and the FUSION FX7™ system (B).

FUSION™ Multi-Imaging

FUSION FX7™ Advance
Cat. No.: 60-FU-26MXA

FUSION SL™ Advance
Cat. No.: 60-FU-SLA

FUSION Solo™
Cat. No.: 60-FU-SOLO

FUSION Xpress™
Cat. No.: 60-FU-XP

BIO-1D analysis software
Cat. No.: 60-BIO-1D

AceGlow™ chemiluminescence substrate
Cat. No.: 37-3420

Of course we offer product demos of our imaging systems and software for your lab! Just make an appointment!

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