

Comparative performance of blue film for visualization of proteins

Blue film is routinely used in protein research to obtain fast and qualitative results in chemiluminescent blots. The sensitivity of blue film is lower than that of Amersham™ Hyperfilm™ but it is often adequate for many screening applications. After conducting an initial experiment with blue film, it is normal to use a higher quality film during the final stages to confirm the initial results and generate publication-grade images.

Amersham Hyperfilm Blue is a blue sensitive film for the detection of chemiluminescent signals in different blotting assays. We conducted a comparative performance evaluation of Amersham Hyperfilm Blue with corresponding products from other suppliers at the laboratories of GE Healthcare Life Sciences. We found that Amersham Hyperfilm Blue provided better sensitivity at short exposure times.

Detection of transferrin blots

To demonstrate the performance of Amersham Hyperfilm Blue, we performed Western blots with a two-fold dilution series of transferrin spiked in *E. coli* starting at 2.5 ng down to 0.6 pg with two alternative blue films; CL-XPosure Film from Thermo Scientific and Kodak™ X-OMAT™ Blue (XB) Film from PerkinElmer. We detected transferrin using ECL™ Prime Western Blotting Detection Reagent before exposing the membranes to the films for 1 and 3 min durations using ImageQuant™ LAS 4010.

Figure 1 shows that after an exposure of 1 min, the film produced a high limit of detection of 9.8 pg.

Figure 2 compares the volume intensity at 1 min exposure for the first four bands of transferrin. Amersham Hyperfilm Blue film took up the signal more efficiently after 1 min film exposure compared with CL-XPosure film and Kodak X-OMAT Blue (XB) Film.

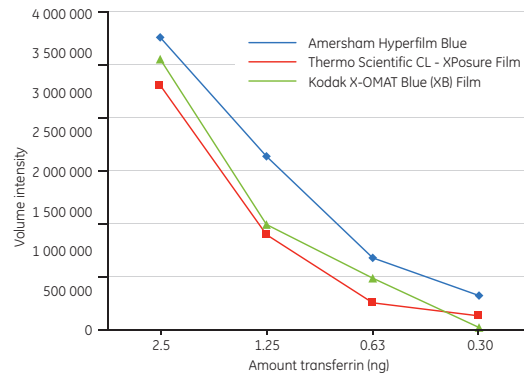


Fig 2. Volume intensity calculated with ImageQuant TL 7.0 software from the first four bands of transferrin, 2.5 ng down to 0.3 ng.

Conclusion

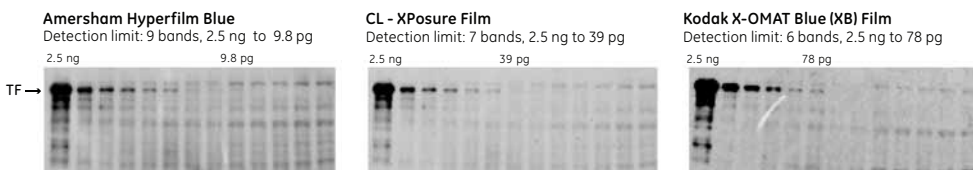
The data shows that Amersham Hyperfilm Blue film was more sensitive after 1 min exposure compared to blue film from Thermo Scientific and PerkinElmer.

Ordering information

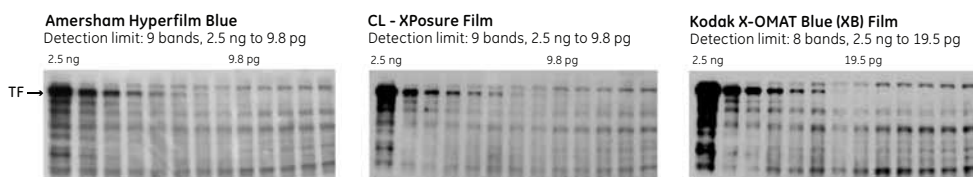
Product	Code number
Amersham Hyperfilm Blue 5 × 7 inches, 100 sheets	28-9888-22
Amersham Hyperfilm Blue 18 × 24 cm, 100 sheets	28-9888-21
Amersham Hyperfilm Blue 8 × 10 inches, 100 sheets	28-9888-20

For more information, visit www.gelifesciences.com/hyperfilm

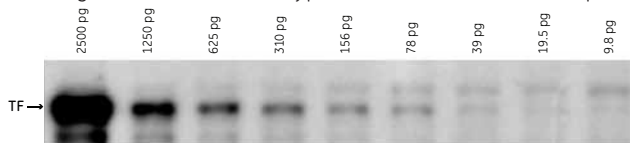
A) 1 min film exposure



B) 3 min film exposure



C) Enlargement of Amersham Hyperfilm Blue after 1 min film exposure



Experimental conditions

Sample load:	Two-fold dilution series of transferrin from 2.5 ng spiked in <i>E. coli</i>
Gel:	Invitrogen™ Novex™ 12% Tris-Glycine gel, 1.0 mm × 15 well
Marker:	High-Range Rainbow™ Molecular Weight Marker
Membrane:	Hybond™ LFP PVDF Transfer membrane
Blocker:	3% BSA
Primary Ab:	Polyclonal rabbit anti-human transferrin (DakoCytomation) dilution 1:4000
Secondary Ab:	ECL Rabbit IgG, HRP-Linked Whole Ab dilution 1:40000
Detection reagent:	ECL Prime Western Blotting Detection Reagent
Film detection:	Amersham Hyperfilm Blue Thermo Scientific CL-XPosure Film Kodak X-OMAT Blue (XB) Film
Imaging:	ImageQuant LAS 4010

Fig 1. A) and B) Performance of Amersham Hyperfilm Blue, CL-XPosure Film (Thermo Scientific) and Kodak X-OMAT Blue (XB) Film (PerkinElmer) with Western blots of a two-fold dilution series of transferrin, from 2.5 ng, spiked in *E. coli*. Transferrin was detected using ECL Prime Western Blotting Detection Reagent. The films were exposed to the membrane for 1 and 3 min. **C)** Enlargement of Amersham Hyperfilm Blue after 1 min exposure. Amersham Hyperfilm Blue illustrates a high limit of detection, 9.8 pg. TF=Transferrin.