

- High signal-tobackground ratio enables protein detection down to the subnanogram range using fluorescent western blotting
- Compatible detection methods:
  - Colorimetric
  - Chemiluminescent
  - Fluorescent
  - Chemifluorescent
- Key characteristics: low autofluorescence, high protein-binding capacity, low nonspecific binding, and durability



# Superior Membranes for Your Fluorescent Immunoblotting Needs

With recent technological advances, fluorescent detection is becoming more mainstream given the unique advantages it provides over chemiluminescent detection; these include a longer signal duration, broader dynamic range, more accurate quantitation, and the ability to multiplex.

Polyvinylidene fluoride (PVDF) membranes are a popular choice for immunoblotting applications because of their key characteristics, which include durability, low non-specific binding, high sensitivity, and the ability to withstand reprobing. However, standard PVDF membranes are autofluorescent and therefore generate a high background and lower signal-to-background ratio, reducing dynamic range and making accurate quantitation more challenging.

Bio-Rad's Immun-Blot LF PVDF membranes offer the same benefits as standard PVDF membranes in addition to minimal autofluorescence and high sensitivity, making them the premium choice for fluorescent and multiplex fluorescent immunoblotting needs.

## **High Signal-to-Background Ratios**

Fluorescence applications require highquality membranes without the inherent autofluorescence of standard PVDF membranes. Bio-Rad's Immun-Blot LF PVDF membranes produce highly sensitive and quantitative results (Figure 1) because the fluorescent blots exhibit superior signal-tobackground ratios (Figure 2).

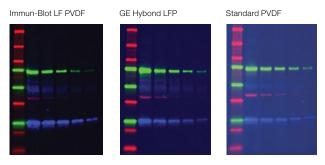


Fig. 1. Immun-Blot LF PVDF membranes have minimal autofluorescence compared to other PVDF membranes.

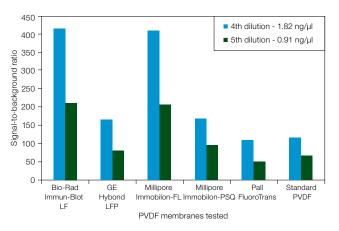


Fig. 2. Comparison of signal-to-background ratios from the fourth and fifth dilutions. Signal-to-background ratios were plotted to demonstrate increased signal detection due to the low autofluorescent properties of some of the PVDF membranes tested. Analysis was performed on the blue channel since this is where autofluorescence is most common and problematic.



## **High Sensitivity**

The superior binding capacity of the membranes allows them to retain more proteins, thus improving the transfer efficiency and downstream detection of low abundance proteins. The improved sensitivity of Bio-Rad's LF PVDF membranes in comparison to other PVDF membranes is demonstrated in Figure 3. The serial dilution of GST was performed, processed, and imaged simultaneously.

Immun-Blot LF PVDF 500 ng	0.45 ng	Standard PVDF 500 ng	0.45 ng
Millipore Immobilon-P 500 ng	0.45 ng	Millipore Immobilon-FL 500 ng	0.45 ng

Fig. 3. Immun-Blot LF PVDF membranes provide higher sensitivity than other PVDF membranes on the market.

#### Table 1. Comparison of pore size and binding capacity of low fluorescent membranes.

Vendor	Bio-Rad Immun-Blot LF PVDF	Pall FluoroTrans PVDF	GE Healthcare Hybond LFP	Millipore Immobilon-FL	Millipore Immobilon-P
Pore size, µm	0.45	0.22	0.22	0.45	0.45
Binding capacity, µg/cm <sup>2</sup>	300	300	>400	300	294

#### Table 2. Common fluorescent stains and dyes.

			Filters	
Fluorescent Stains/Dyes	Light Sources	Pharos FX <sup>™</sup>	ChemiDoc™ MP	ChemiDoc <sup>™</sup> XRS+
Cy2	Epi Blue	530DF30	530/28	520/30
СуЗ	Epi Green	605BP	605/50	Not Supported
Cy5	Epi Red	695DF55	695/55	Not Supported
Alexa Fluor 488	Epi Blue	530DF30	530/28	520/30
Alexa Fluor 532	Epi Green	605BP	530/28	Not Supported
Alexa Fluor 546	Epi Green	605BP	605/50	Not Supported
Alexa Fluor 555	Epi Green	605BP	605/50	Not Supported
Alexa Fluor 633	Epi Red	695DF55	695/55	Not Supported
Alexa Fluor 647	Epi Red	695DF55	695/55	Not Supported
Alexa Fluor 680	Epi Red	695DF55	695/55	Not Supported
DyLight 488	Epi Blue	530DF30	530/28	520/30
DyLight 549	Epi Green	605BP	605/50	Not Supported
DyLight 633	Epi Red	695DF55	695/55	Not Supported
DyLight 649	Epi Red	695DF55	695/55	Not Supported
DyLight 680	Epi Red	695DF55	695/55	Not Supported
Qdot 525	488 nm / UV Trans	530DF30	530/28	520/30
Qdot 565	488 nm / UV Trans	555LP	530/28	560/50 or std
Qdot 585	488 nm / UV Trans	Not Supported	630/50	560/50 or std
Qdot 605	488 nm / UV Trans	605BP	630/50	std
Qdot 625	488 nm / UV Trans	605BP	630/50	630/30 or std
Qdot 655	488 nm / UV Trans	695DF55	695/55	630/30 or std
Qdot 705	488 nm / UV Trans	695DF55	695/55	Not Supported

### **Ordering Information**

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162-0260Immun-Blot LF PVDF Filter Sets, pkg of 10, 7 x 8.5 cm, precut low fluorescence PVDF/filter paper for immunoblotting162-0261Immun-Blot LF PVDF Filter Sets, pkg of 20, 7 x 8.5 cm, precut low fluorescence PVDF/filter paper for immunoblotting162-0262Immun-Blot LF PVDF Filter Sets, pkg of 10, 8.5 x 13.5 cm, precut low fluorescence PVDF/filter paper for immunoblotting162-0263Immun-Blot LF PVDF Filter Sets, pkg of 20, 8.5 x 13.5 cm, precut low fluorescence PVDF/filter paper for immunoblotting162-0263Immun-Blot LF PVDF Filter Sets, pkg of 20, 8.5 x 13.5 cm, precut low fluorescence PVDF/filter paper for immunoblotting162-0264Immun-Blot LF PVDF Membrane, pkg of 1 roll, 28 cm x 3.8 m, low fluorescence PVDF membrane for immunoblotting

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