



Figure 24.49 Molecular Probes' 75 mm × 75 mm gelatin photographic filters for use with Polaroid black-and-white print film photography.

Molecular Probes has by far the best fluorescent stains for both nucleic acid and protein gels and blots. Our SYBR, SYPRO, PRO-Q, Amplex Gold and DyeChrome products are described in Chapters 8 and 9.

24.4 Photographic Filters for Electrophoretic Gels and Blots

Molecular Probes offers a number of fluorescent reagents for staining nucleic acids and proteins in gels and on blots, described in Section 8.4, Section 9.3 and Section 9.4. Preeminent among these stains are our SYBR Green and SYBR Gold nucleic acid gel stains (Section 8.4) and our SYPRO protein stains for gels and blots (Section 9.3, Section 9.4) as well as our DyeChrome, Amplex Gold and Pro-Q Western Blot Stain Kits, Pro-Q Oligohistidine Stain Kits and reagents and our Pro-Q Glycoprotein Stain Kits (Section 9.4). To achieve optimal sensitivity with these exceptional fluorescent dyes, it is *essential* to photograph the gel or blot because the camera's integrating capability can make bands visible that are not detected by eye. Photographs should be taken using a photographic filter with spectral properties closely matched to those of the fluorescent dye used. Molecular Probes provides several 75 mm × 75 mm gelatin filters (Figure 24.49) optimized for photographing stained gels or blots with a Polaroid camera and Polaroid 667 black-and-white print film. Note that these gelatin filters are generally not suitable for use with portable or stationary gel-documentation systems or with CCD cameras.

SYBR Photographic Filter

To achieve optimal sensitivity using Polaroid 667 black-and-white print film and UV illumination, DNA or RNA gels stained with our proprietary SYBR Green I, SYBR

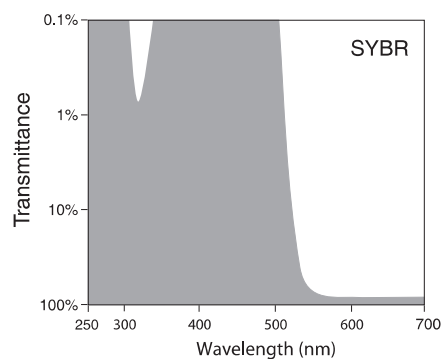


Figure 24.50 Transmittance profile of the SYBR photographic filter (S-7569).

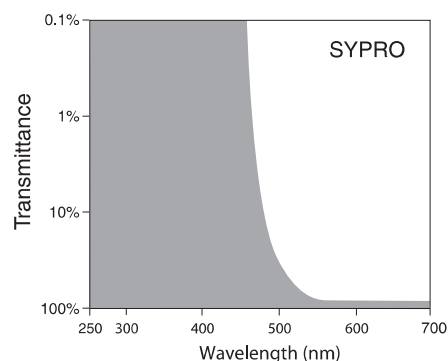


Figure 24.51 Transmittance profile of the SYPRO photographic filter (S-6656).

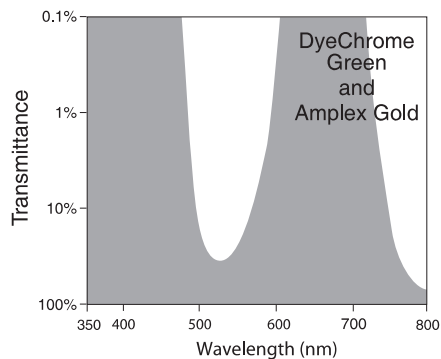


Figure 24.52 Transmittance profile of the DyeChrome Green photographic filter (part of D-24771) and Amplex Gold photographic filter (A-24772).

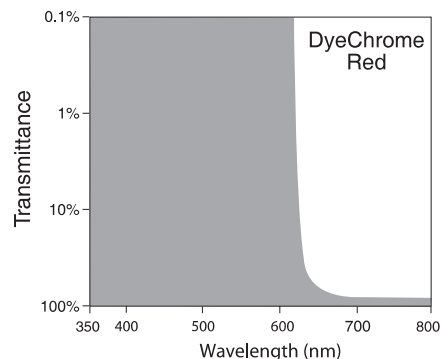


Figure 24.53 Transmittance profile of the DyeChrome Red photographic filter (part of D-24771).

Green II or SYBR Gold nucleic acid gel stains (Section 8.4) should be photographed through the SYBR photographic filter (S-7569, Figure 24.50). The SYBR photographic filter is also recommended for photographing Southern blots or dot blots stained with our SYBR DX DNA blot stain (S-7550, Section 8.5).

SYPRO Photographic Filter

To achieve optimal sensitivity using Polaroid 667 black-and-white print film and UV illumination, protein gels or blots stained with any of our proprietary SYPRO protein stains (including the SYPRO Orange, SYPRO Red, SYPRO Tangerine and SYPRO Ruby protein gel stains and the SYPRO Ruby and SYPRO Rose Plus protein blot stains) should be photographed through our SYPRO photographic filter (S-6656, Figure 24.51). The SYPRO photographic filter is also ideal for photographing DDAO, used in some of our Western Blot Stain Kits, Glycoprotein Stain Kits and Oligohistidine Blot Stain Kits, which are described in Section 9.4. This filter can also be used with our Pro-Q Sapphire oligohistidine gel stains, which are also described in Section 9.4.

DyeChrome Red/Green Photographic Filter Set

The use of photographic filters to document fluorescent staining patterns not only maximizes sensitivity, but also provides an

opportunity to separate multiple fluorescent signals on the same blot. Our DyeChrome Red/Green Photographic Filter Set (D-24771) is optimized for nearly flawless separation of the red and green fluorescent signals produced by the reagents in the DyeChrome Western Blot Stain Kits (Section 9.4, Figure 24.52, Figure 24.53), while maintaining excellent sensitivity. Use of this filter set makes it possible to perform sophisticated multicolor experiments without the need to invest in expensive digital imaging devices or laser-based scanning instruments. The filter set works equally well for separating DDAO and BODIPY FL-X signals (products of the reagents used in the DyeChrome Western Blot Stain Kits #1, #2 and #3) or ELF 39 alcohol and BODIPY TR-X signals (products of the reagents used in the DyeChrome Western Blot Stain Kits #4, #5 and #6).

Amplex Gold Photographic Filter

The Amplex Gold photographic filter (A-24772), which has the same transmittance profile as the “green” filter used in the DyeChrome Red/Green Photographic Filter Set (Figure 24.52) is optimal for detecting the fluorescence of the oxidized Amplex Gold horseradish peroxidase substrate, which is used in our Amplex Gold Western Blot Stain Kits #1, #2 and #3 (Section 9.4) and the DyeChrome Double Western Blot Stain Kit (D-21887, Section 9.4).

Product List — 24.4 Photographic Filters for Electrophoretic Gels and Blots

Cat #	Product Name	Unit Size
A-24772	Amplex® Gold photographic filter	each
D-24771	DyeChrome™ Red/Green Photographic Filter Set *two filters*	1 set
S-7569	SYBR® photographic filter	each
S-6656	SYPRO® photographic filter	each

24.5 Optical Filters for Fluorescence Microscopy

Sensitive and versatile fluorescence detection techniques are of ever-increasing importance and popularity in biological research microscopy (Fluorescence Microscopy, Second Edition. B. Herman. Bios Scientific Publishers (1998); available from Molecular Probes, F-14942, Section 24.6). In the now-standard epi-illuminated microscope configuration, the optical filter set performs a critical function in separating the fluorescence emission photons that will form the final image from the more-intense excitation light field. For practical purposes, it is necessary to reduce the excitation light intensity in the detection path by a factor of 10^6 – 10^7 . This design objective has to be achieved in parallel with capturing as many of the available fluorescence photons as possible. High capture efficiency allows compensating reductions in overall excitation light levels, with accompanying reductions in dye photobleaching and cellular phototoxicity.

The Optical Filter Set

A set of optical filters for selective excitation and detection of fluorescence typically consists of a minimum of three components: an excitation filter, a dichroic beamsplitter (“dichroic mirror”) and an emission filter (“barrier filter”) (Figure 24.54). The excitation

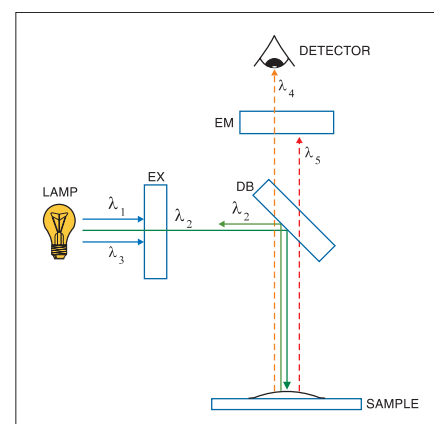


Figure 24.54 Functions of fluorescence microscope filter set components. The desired excitation wavelength (λ_2) is selected from the spectral output of the lamp by the excitation filter (EX) and directed to the sample via the dichroic beamsplitter (DB). The beamsplitter separates emitted fluorescence (....) from scattered excitation light (—). The emission filter (EM) selectively transmits a portion of the sample's fluorescence emission (λ_4) for detection and blocks other emission components (λ_5).