Excitation and Emission of Green Fluorescent Proteins

Green fluorescent proteins are being used for more and more applications in molecular and cellular biology. As a result of the variety of applications several variants form the original wild type green fluorescent protein (wtGFP) have been developed. Several of these variants have different excitation and emission spectra than wtGFP. In order for maximal sensitivity to be obtained when quantitating these GFPs it is important that the most appropriate filter set be used. Here we characterize the excitation and emission spectra of some of the commonly used variants of GFP and recommend appropriate filter sets for detection and quantitation using the FL600 microplate fluorescence reader.

In terms of filter sets for the FL600 microplate fluorescence reader, the most commonly used mutations to the wtGFP protein can be categorized into three groups: (1) the red-shifted variants; (2) the wild type like variants; and (3) the blue emitting variants. As demonstrated in Figure 1, the red-shifted variants, typified by EGFP, have a single excitation peak centered at about 488 nm, with an emission peak wavelength of 509 nm. The wild type like variants have their primary excitation peak centered on 395 nm, with an emission peak at 509 nm while the blue emitting mutants generally have an excitation peak at around 380 nm and an emission peak near 460 nm (Figure 1).

Table 1. Excitation and emission data of GFP variants

GFP variant	Excitation max (nm)	Emission max (nm)	Excitation Filter[@]	Emission Filter[@]
wtGFP	395	509	400/30[#]	508/20
			360/40	
GFPuv	395	509	400/30[#]	508/20
			360/40	
Stemmer	395	509	400/30[#]	508/20
			360/20	
EGFP	488	509	485/20	530/25
GFP-S65T	489	509	485/20	530/25
Y66H	382	459	360/40	460/40
EBFP	380	460	360/40	460/40

@ Note that the center wavelength and bandpass are indicated.
# Note that the 400/30 excitation is recommended as being slightly superior

Table 1 indicates the possible filter sets that would be recommend for the use of the FL600 in measuring the fluorescence of different GFP variants. Note that although the peak for excitation of wtGFP, GFPuv, and the Stemmer mutant is closer to the 400/30 excitation filter, the 360/40 filter that is a standard filter for the FL600 provides quite adequate fluorescent signal.

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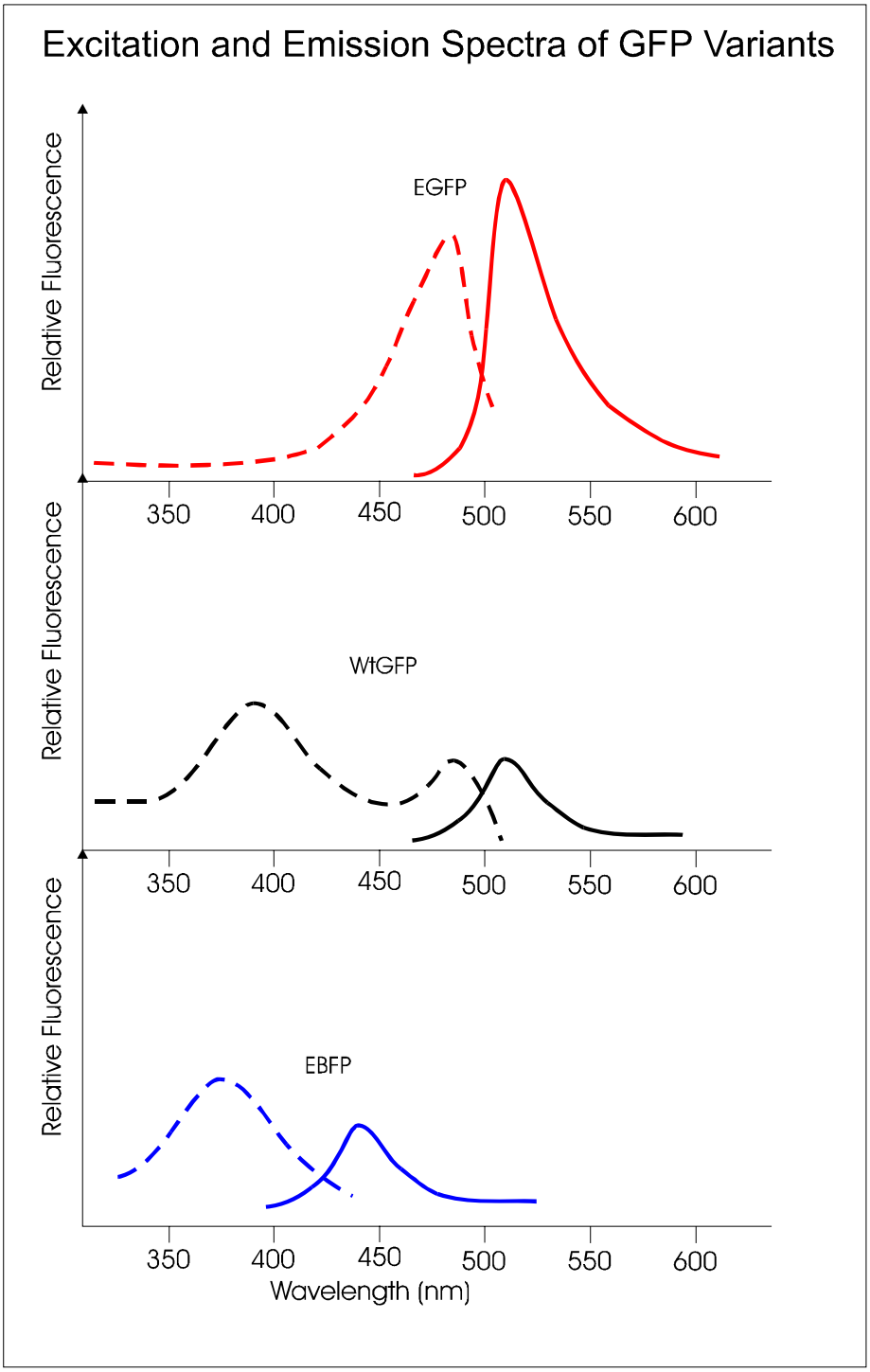


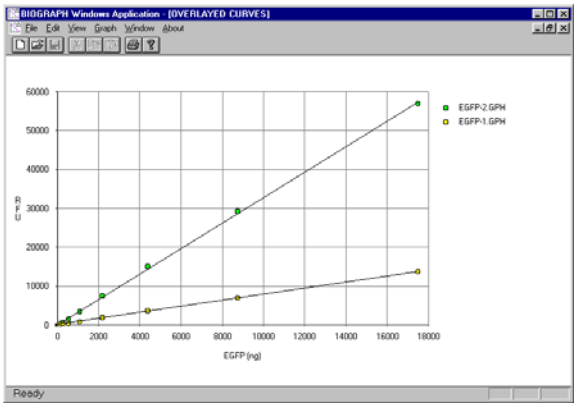
Figure 1. Excitation and emission spectra of EGFP, wtGFP, and EBFP. Representative excitation (dashed lines) and emission (solid lines) spectra of the three basic GFP variants. Data was derived from reported spectra in the literature.

The red-shifted mutants have been developed for use with the standard “fluorescein” filter set and as such utilize the 485/20 excitation and 530/25 emission filter set. Note that although the 508/20 emission filter is closer to the emission peak of the sample, overlap with the 485/20 filter precludes the use of that filter, necessitating the use of the 530/25 nm filter. The blue emitting

variants generally require the use of the 360/40 excitation filter in conjunction with the 460/40 emission filter.

As noted above, many of the GFP variants utilize filters that are provided standard with the FL600. If wtGFP is being measured, it is recommended that the 400/20 excitation filter be used in conjunction with a 508/20 emission filter, both of which are custom filters stocked by BioTek Instruments. Generally acceptable data can be obtained if the 360/40 excitation filter is used, but the 508/20 emission filter is definitely required. The red-shifted mutants generally only require the standard "fluorescein" filter set of the 485/20 excitation and 530/25 emission, both of which are standard filters on the FL600. Likewise, the blue emitting mutants use the 360/40 excitation and 460/40 emission filters, also standard on the FL600.

A



B

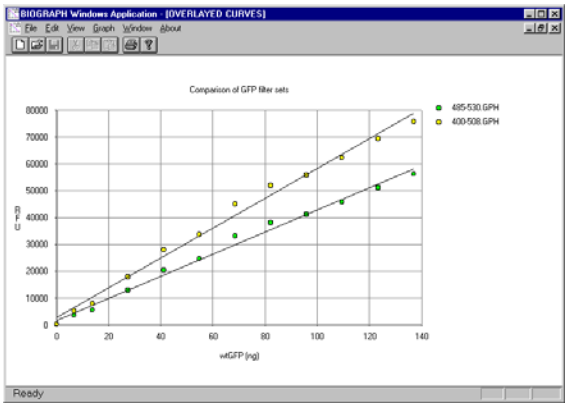


Figure 2. Loss of signal when using alternative filter sets. Dilutions of (A) EGFP protein or (B) wtGFP were made using 10 mM Tris, 10 mM EDTA buffer as the diluent. Samples were read using an FL600 fluorescence plate reader with reader function controlled by KC4 data reduction software on an external PC. Fluorescence was determined using either a 485/20 excitation, 530/25 emission filter set or a 400/30 excitation, 508/20 emission filter set. For EGFP and wtGFP determinations using either filter set, a sensitivity setting of 170 was used.

As demonstrated in Figure 2, it is critical that the appropriate filter set be utilized for the GFP variant being measured. Using the "fluorescein" filter set (485/20 excitation, 530/25 emission) when quantitating wtGFP results in a 20% loss of signal, when compared to the recommended 400/30 excitation, 508/20 emission filter set (Figure 2B). Likewise, a much larger drop in signal (75%) is observed when the fluorescence of EGFP is determined with the 400/30 excitation, 508/20 emission filter set rather than its recommended filter set of 485/20 excitation, 530/25 emission filters (Figure 2A). A similar drop in signal would be expected if the blue emitting GFPs, such as EBFP, are quantitated using the inappropriate filter set (data not shown). These data are in close agreement with the excitation and emission spectra of the proteins portrayed in Figure 1.