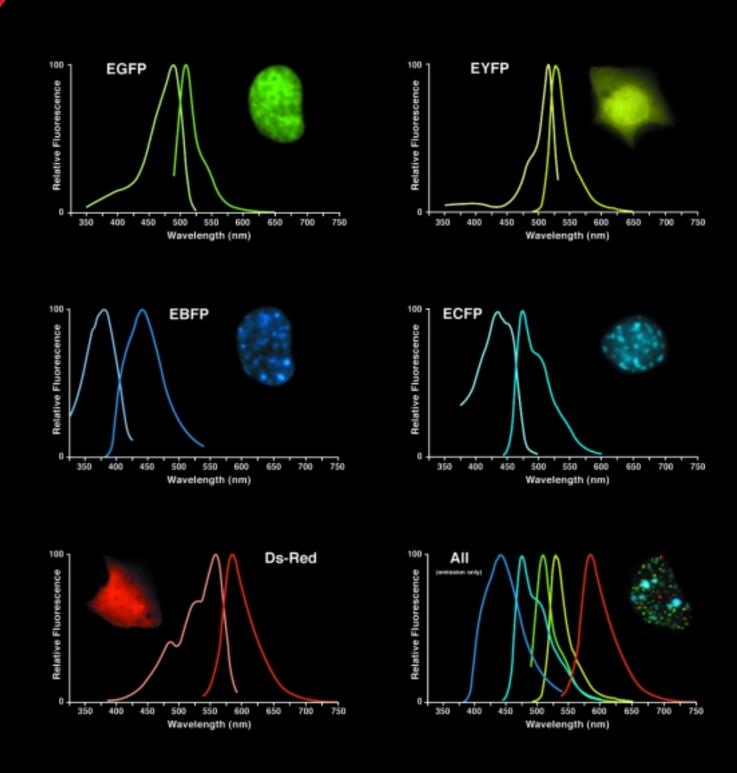


GFP 3.1



Handbook of Filter Sets for Fluorescent Proteins



Chroma Technology is an employee owned company that specializes in the design and manufacture of precision optical filters and coatings. Our filters are designed for a variety of applications, including fluorescence microscopy, which require the greatest accuracy in color separation, optical quality, and signal purity.

The GFP brochure describes most of the filter sets that we have developed for viewing and imaging the various GFP mutants. For each filter set we have provided a spectral trace and, when applicable, a note on the unique quality of that set.

Inside the back cover you will find a pocket which contains application notes. We will forward additional material as collected.

Chroma's filter designs will keep pace with the rapidly emerging applications. Please contact us if our filter set lists do not contain the combination that you require.

For the most current information please visit our web site www.chroma.com.



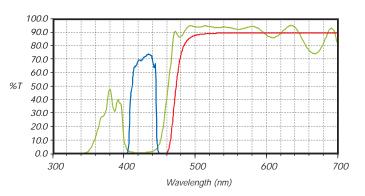
Cover Image: Fluorescent Protein Spectra by George Patterson, Rich N. Day and David Piston, Courtesy Journal of Cell Science 2001 (114, pp. 837-838)

Inside Cover Image: Dr. Steve Kay and Dr. Jeff Plautz, University of Virginia



11003 wtGFP (longpass emission)

| exciter | D425/40 |
|----------|---------|
| dichroic | 460DCLP |
| emitter | GG475LP |

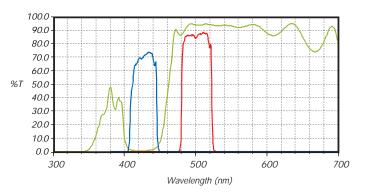


Violet excitation with longpass emission. Shorter wavelength excitation, e.g. UV and Violet, may increase photobleaching.

31019 wtGFP

(bandpass emission)

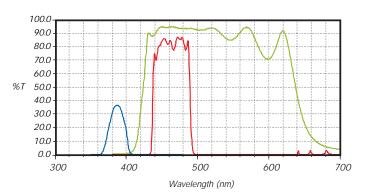
| exciter | D425/40 |
|----------|---------|
| dichroic | 460DCLP |
| emitter | D500/40 |



A bandpass emission filter is especially important when using GFP with a second and longer wavelength fluorochrome.

31021 Blue GFP

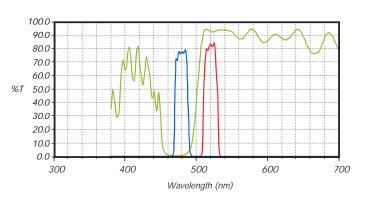
| exciter | D390/22 |
|----------|---------|
| dichroic | 420DCLP |
| emitter | D460/50 |



One of two Chroma BFP filter sets. When testing the two versions, the narrow excitation filter was preferred by Dr. Steve Kay of Scripps Research Institute, whom we have worked with on other GFP filter sets. The narrow excitation filter avoids 365nm excitation of NADH.

31026 Narrow band excitation and emission

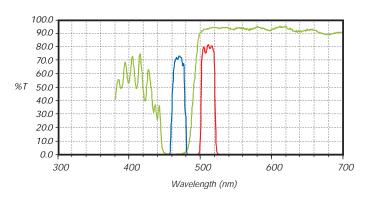
| exciter | D480/20 |
|----------|---------|
| dichroic | 490DCLP |
| emitter | D520/20 |



This filter set was an early attempt at eliminating autofluorescence with the use of narrow band excitation and emission filters.

31039 JP1(for EGFP when used with YGFP)

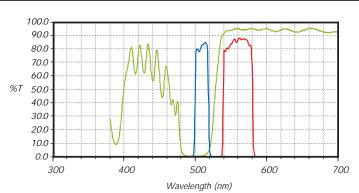
| exciter | D470/20 |
|----------|---------|
| dichroic | 490DCLP |
| emitter | D510/20 |



The spectral characteristics of this filter set were described by Dr. Jonathon Pines of the University of Cambridge. Dr. Pines has used this filter set, together with filter set 31040 (JP2), for dual imaging EGFP and EYFP.

31040 JP2 (for YGFP when used with EGFP)

| exciter | D510/20 |
|----------|---------|
| dichroic | 530DCLP |
| emitter | D560/40 |

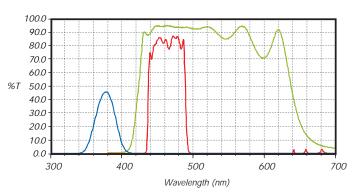


The companion filter set for 31039. To view YFP and exclude GFP, a subtraction algorithm is required.



31041 Blue GFP II

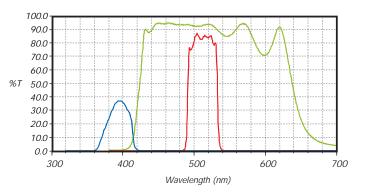
| exciter | D380/30 |
|----------|---------|
| dichroic | 420DCLP |
| emitter | D460/50 |



The second BFP filter set. While testing these filter sets we found that scientists at Clontech preferred the wider excitation filter and recommended this set to their customers.

31043 Sapphire/UV GFP

| exciter | D395/40 |
|----------|---------|
| dichroic | 425DCLP |
| emitter | D510/40 |

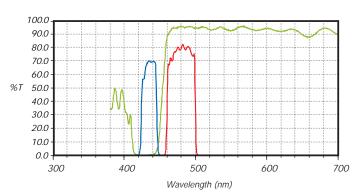


Sapphire GFP filter set. We have not seen a lot of interest in this GFP mutant as yet. Its narrow emission band and UV excitation do not seem to make it a good candidate for dual staining with other GFPs. It might be used as a dual label with YFP, if the YFP does not excite in the UV.

31044v2

| C٧ | /an | G | F | P |
|----|-----|---|---|---|
| | | | | |

| exciter | D436/20 |
|----------|---------|
| dichroic | 455DCLP |
| emitter | D480/40 |



Cyan GFP filter set. Welldesigned for dual staining with EYFP. Problematic for dual staining with EGFP, as it will likely excite the EGFP.

R TECHNOLOGY C O R P

32000 wtGFP (bandpass emission) 100.0 D425/60 90.0 470DCXR 80.0 70.0 D510/40 60.0 %Т 50.0 40.0 30.0 20.0 10.0 0.0

400

300

Our original wtGFP filter set. The wide excitation attempted to capture the absorption spectrum of the wtGFP. Short wavelength excitation may increase photobleaching.

32001 wtGFP

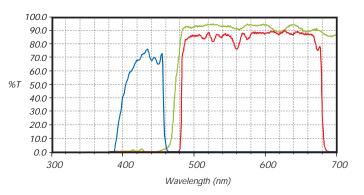
exciter

dichroic

emitter

(longpass emission)

| D425/60 |
|---------|
| 470DCXR |
| E480LP |
| |



500

Wavelength (nm)

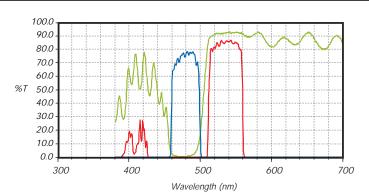
600

700

The longpass version of filter set 32000.

41001 FITC/EGFP (bandpass emission)

| exciter | HQ480/40 |
|----------|----------|
| dichroic | Q505LP |
| emitter | HQ535/50 |

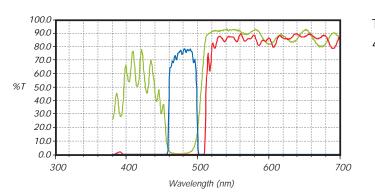


Our original High Q FITC filter set. It also works well with EGFP. This set maximizes the excitation signal but cuts off the short half of the emission spectrum.



41012 FITC/EGFP (longpass emission)

| exciter | HQ480/40 |
|----------|----------|
| dichroic | Q505LP |
| emitter | HQ510LP |

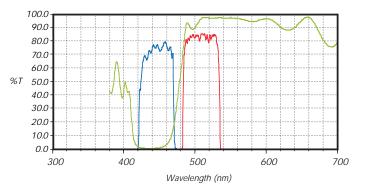


The longpass version of filter set 41001.

41014 wtGFP

(bandpass emission)

| exciter | HQ450/50 |
|----------|----------|
| dichroic | Q480LP |
| emitter | HQ510/50 |

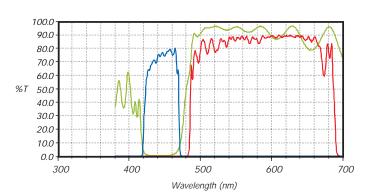


In response to the problems with photobleaching, we switched excitation to the second absorption peak of wtGFP. This filter set was designed to capture most of the fluorescence signal while sacrificing some of the excitation light. This set is better for the wtGFP but also works with EGFP.

41015 wtGFP

(longpass emission)

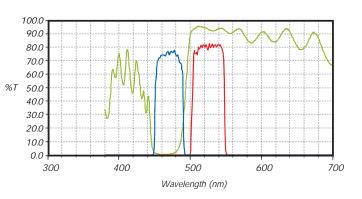
| exciter | HQ450/50 |
|----------|----------|
| dichroic | Q480LP |
| emitter | HQ485LP |



The longpass version of set 41014.

41017 Endow GFP (bandpass emission)

| exciter | HQ470/40 |
|----------|----------|
| dichroic | Q495LP |
| emitter | HQ525/50 |

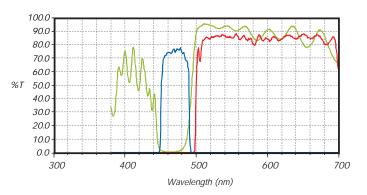


The spectral characteristics of this filter set were specified by Dr. Sharyn Endow of Duke University. Compared to filter set 41014, it captures more of the excitation light while sacrificing some of the fluorescence signal. This set is excellent for both the wtGFP and EGFP. It is our most widely distributed GFP filter set (see Endow and Korma, *J Cell Biology* 137, 1321-1336, 1997).

41018 Endow GFP

(longpass emission)

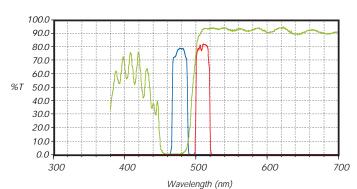
| exciter | HQ470/40 |
|----------|----------|
| dichroic | Q495LP |
| emitter | HQ500LP |
| | |



The longpass version of filter set 41017.

41020 Narrow band excitation and emission

| exciter | HQ480/20 |
|----------|----------|
| dichroic | Q495LP |
| emitter | HQ510/20 |



An early High Q attempt at eliminating auto-fluorescence with use of narrow band excitation and emission filters.



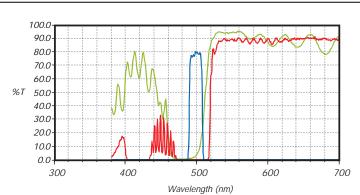
41025 **Piston GFP** 100.0 HQ470/40 The spectral characteristics of this exciter 90.0 filter set were specified by Dr. David Q495LP dichroic 80.0 70.0 Piston of Vanderbilt University. The emitter HQ515/30 60.0 narrow emission filter was designed to %Т 500 40.0 quantitate the EGFP fluorescence 30.0 while avoiding auto-fluorescence at 20.0 10.0 wavelengths longer than 540nm 0.0 (Patterson, G.H., S.M. Knobel, W.D. 300 400 500 600 700 Sharif, S.R. Kain, D.W. Piston. "Use of Wavelength (nm) the Green Fluorescent Protein (GFP) and its Mutants in Quantitative Fluorescence Microscopy." Biophysical 41028 Yellow GFP Journal 73: 2782-2790, 1997.) 100.0 High Q bandpass emission filter 90.0

80.0 70.0 60.0 %Т 50.0 40.0 30.0 20.0 10.0 0.0 300 400 500 600 700 Wavelength (nm)

set for EYFP. Well-designed for dual staining with CFP.

41029 Yellow GFP (longpass emission)

| exciter | HQ500/20 |
|----------|----------|
| dichroic | Q515LP |
| emitter | HQ520LP |



The longpass version of set 41028.

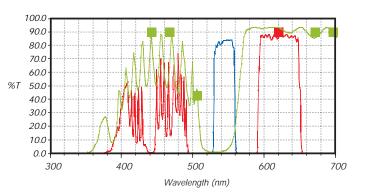
| 41020 | TCHOV | |
|-------|---------|----------|
| (band | pass er | nission) |

| exciter | HQ500/20 |
|----------|----------|
| dichroic | Q515LP |
| emitter | HQ535/30 |



DsRed exciter HQ545/30 dichroic Q570LP

HQ620/60



A variety of filter sets will work for red fluorescent protein. This set is best for visualizing DsRed.

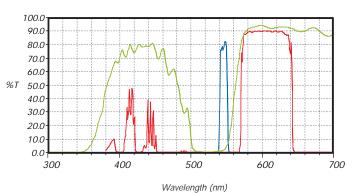
41035

41002c

emitter

DsRed

| exciter | HQ546/12 |
|----------|----------|
| dichroic | Q560LP |
| emitter | HQ605/75 |



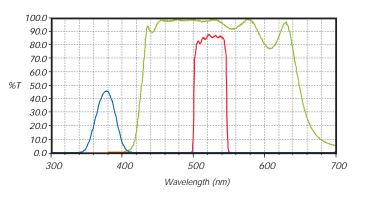
Custom narrow band excitation set designed for very specific applications with DsRed.



31034 BFP/GFP FRET (BP)

| exciter | D380/30 |
|----------|---------|
| dichroic | 425DCLP |
| emitter | D525/50 |

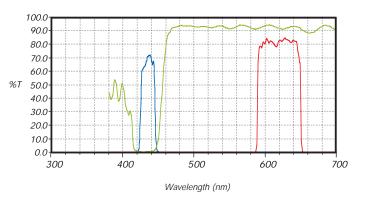
The sets listed below are examples of sets that can be used for fluorescence resonance energy transfer. There are other sets that can be used. Please call for specifications of other sets.



BFP and GFP were the original fluorescent protein FRET pair. Not as widely used due to the photoinstability of the BFP and the need to excite living cells in the UV. This is the bandpass/ wide excitation version of our BFP/GFP FRET sets. We have several additional BFP/GFP FRET pairs. Call for more details.

31051 CFP/DsRed FRET

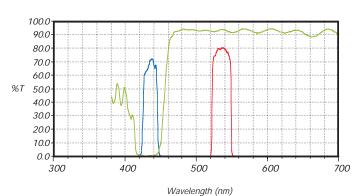
| exciter | D436/20 |
|----------|---------|
| dichroic | 455DCLP |
| emitter | D620/60 |



CFP and DsRed should prove to be a useful FRET pair. There is significant overlap between the emission wavelengths of CFP and broad excitation range of DsRed. Additionally, the red emission of the DsRed should be well separated from the broad emission tail of CFP, allowing for a fairly clean FRET signal.

31052 CFP/Topaz FRET

| exciter | D436/20 |
|----------|---------|
| dichroic | 455DCLP |
| emitter | D535/30 |



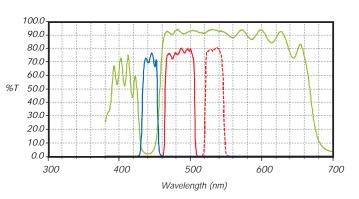
Our standard FRET set for use with CFP and EYFP. The broad emission tail of CFP requires that ratio imaging be performed to identify the FRET signal. Call for details.

R(TECHNOLOGY C O R P

71007a

Cameleons 2

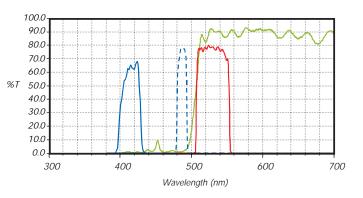
| exciter | D440/20 |
|----------|---------|
| dichroic | 455DCLP |
| emitter | D485/40 |
| emitter | D535/30 |



Chroma's revision of the filter set described by Dr. Roger Tsien for Cameleons 2 FRET (see Miyawaki, et al, Nature 388, pp. 882-887, 1997). Depending on the hardware available, there may be the need for an emission separating beamsplitter.

71009 LS/P Cyan/Topaz dual excitation single emission

| exciter | D414/30 |
|--------------------|----------|
| <pre>exciter</pre> | HQ487/15 |
| dichroic | 505DCXR |
| emitter | HQ530/50 |



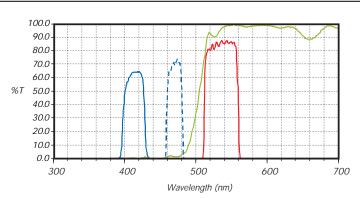
This filter set was specified by Dr. John Presley from the lab of Dr. Jennifer Lippincott-Schwartz. The excitation filters were designed to excite one of the proteins without exciting the other. This allows BioTechniques 25, pp. 838-846

one to capture each image sequentially and overlay the results (see Ellenberg, et al, November, 1998).

A custom filter set designed to image the changes in spectral characteristics of pH-sensitive GFP mutants. This is a dual excitation, single emission set requiring hardware for the dual excitation protocol (see Miesenbock, et al, Nature, 394, July 1998).

71012 pH Sensitive GFP

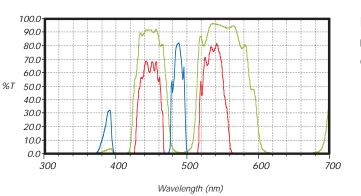
| exciter | D410/30 |
|----------|----------|
| exciter | HQ470/20 |
| dichroic | 500DCXR |
| emitter | HQ535/50 |





51014 BFP/GFP dual band filter set

| exciter | 51014x |
|----------|---------|
| dichroic | 51014bs |
| emitter | 51014m |

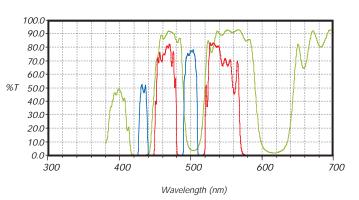


Dual band filter set for simultaneous viewing of Blue and Green GFPs.

51017 CFP/YFP

dual band filter set

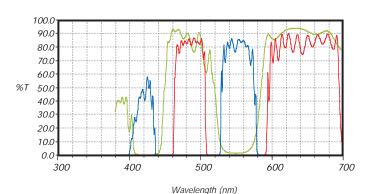
| exciter | 51017x |
|----------|---------|
| dichroic | 51017bs |
| emitter | 51017m |



Dual band filter set for simultaneous viewing of Cyan and Yellow GFPs.

51018 CFP/DsRed dual band filter set

| exciter | 51018x |
|----------|---------|
| dichroic | 51018bs |
| emitter | 51018m |



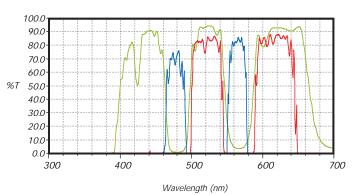
Dual band filter set for simultaneous viewing of Cyan GFP and DsRed.

R TECHNOLOGY C O R P

Dual band filter sets for simultaneous viewing of two GFPs

51019 GFP/DsRed dual band filter set

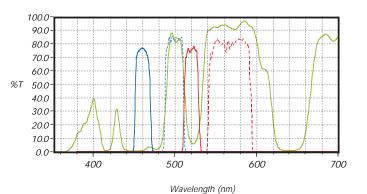
| exciter | 51019x |
|----------|---------|
| dichroic | 51019bs |
| emitter | 51019m |



Dual band filter set for simultaneous viewing of the Green GFP and DsRed.



These filter sets usually require a filter wheel on excitation and emission. The beamsplitter remains stationary while the excitation and emission filters change. The proteins are individually excited and their emissions are captured sequentially.



The spectral characteristics of this filter set were specified by Dr. Jonathon Pines of the University of Cambridge.

86002v1 JP4 for CFP and YFP

86001 JP3 for EGFP

86001bs beamsplitter

S460/20

S523/20

S500/22

S568/50

and YFP

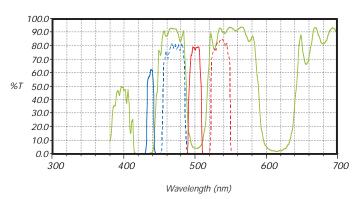
exciter

exciter

emitter

emitter

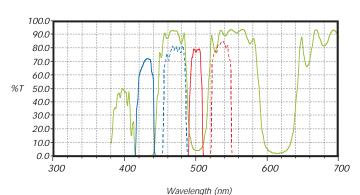
| exciter | S436/10 |
|------------|--------------|
| exciter | S500/20 |
| 86002bs v1 | beamsplitter |
| emitter | S470/30 |
| emitter | S535/30 |



Version 1 is designed for mercury excitation, as the CFP excitation filter is centered directly on a spike in the mercury spectrum and is only 10nm wide.

86002v2 JP4 for CFP and YFP

| exciter | S430/25 |
|------------|--------------|
| exciter | S500/20 |
| 86002bs v2 | beamsplitter |
| emitter | S470/30 |
| emitter | S535/30 |



The excitation filter of version 2 is centered at 430nm and is 25nm wide, which allows this set to be used with either xenon or mercury excitation.

S380/30

S485/25

S445/40

S535/50

These filter sets usually require a filter wheel on excitation and emission. The beamsplitter remains stationary while the excitation and emission filters change. The proteins are individually excited and their emissions are captured sequentially.

100.0 90.0 80.0 70.0 60.0 %Т 500 40.0 30.0 20.0 10.0 0.0 400 300 500 600 700 Wavelength (nm)

Used for the sequential imaging of Blue GFP and Green GFP.

86004 JP5 for UVGFP (Sapphire), CFP and YFP v1

86003bs beamsplitter

86003 for BFP

and EGFP

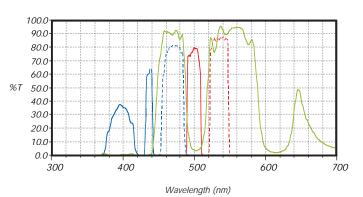
exciter

exciter

emitter

emitter

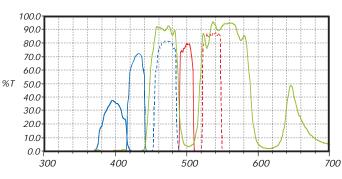
| S395/40 |
|--------------|
| S436/10 |
| S500/20 |
| beamsplitter |
| S470/30 |
| S535/30 |
| |



Version 1 is designed for mercury excitation, as the CFP excitation filter is centered directly on a spike in the mercury spectrum and is only 10nm wide.

86004 JP5 for UVGFP (Sapphire), CFP and YFP v2

| exciter | S395/40 |
|------------|--------------|
| exciter | S430/25 |
| exciter | S500/20 |
| 86004bs v2 | beamsplitter |
| emitter | S470/30 |
| emitter | S535/30 |

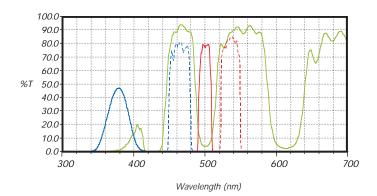


Wavelength (nm)

The excitation filter of version 2 is centered at 430nm and is 25nm wide which allows this set to be used with either xenon or mercury excitation.



These filter sets usually require a filter wheel on excitation and emission. The beamsplitter remains stationary while the excitation and emission filters change. The proteins are individually excited and their emissions are captured sequentially.



Used for the sequential imaging of Blue and Yellow GFP.

86006 CFP/YFP and DsRed

exciter

exciter

emitter

emitter

86005bs beamsplitter

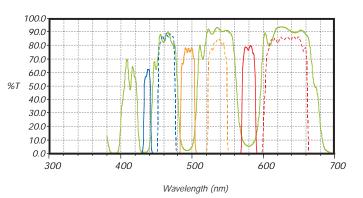
| exciter | S436/10 | |
|----------------------|---------|--|
| exciter | S495/20 | |
| exciter | S580/20 | |
| 86006bs beamsplitter | | |
| emitter | S470/30 | |
| emitter | S535/30 | |
| emitter | S630/60 | |
| | | |

S380/30

S500/20

S465/40

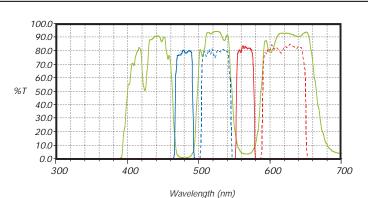
S535/30



Set is designed for mercury or xenon excitation to sequentially visualize Cyan, Yellow and DsRed.

86007 GFP and DsRed

| exciter | S485/25 |
|----------------|--------------|
| exciter | S565/25 |
| 86007bs | beamsplitter |
| emitter | S520/40 |
| emitter | S620/60 |

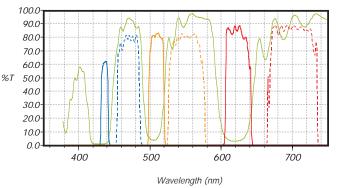


Used for the sequential imaging of the Green GFP and DsRed.

These filter sets usually require a filter wheel on excitation and emission. The beamsplitter remains stationary while the excitation and emission filters change. The proteins are individually excited and their emissions are captured sequentially.

86008v1 CFP/YFP/Cy5

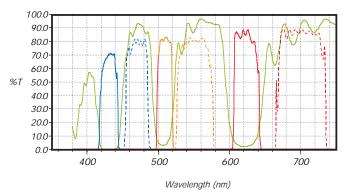
| exciter | S436/10 | |
|----------------------|---------|--|
| exciter | S510/20 | |
| exciter | S622/36 | |
| 86008bs beamsplitter | | |
| emitter | S470/30 | |
| emitter | S550/50 | |
| emitter | S700/75 | |
| | | |



Version 1 is designed for mercury excitation, as the CFP excitation filter is centered directly on a spike in the mercury spectrum and is only 10nm wide.

86008v2 CFP/YFP/Cy5

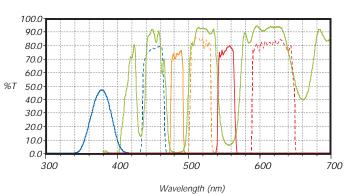
| exciter | S430/25 | |
|----------------------|---------|--|
| exciter | S510/20 | |
| exciter | S622/36 | |
| 86008bs beamsplitter | | |
| emitter | S470/30 | |
| emitter | S550/50 | |
| emitter | S700/75 | |
| | | |



The excitation filter of version 2 is centered at 430nm and is 25nm wide, which allows this set to be used with either xenon or mercury excitation.

86009 BFP/GFP and DsRed

| exciter | S380/30 |
|----------------------|---------|
| exciter | S485/17 |
| exciter | S555/25 |
| 86009bs beamsplitter | |
| emitter | S450/30 |
| emitter | S515/30 |
| emitter | S620/60 |



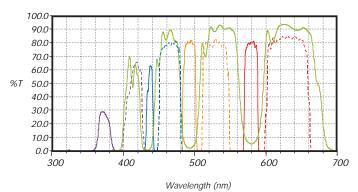
Used for the sequential imaging of the Blue GFP, Green GFP and DsRed.



86010 BFP (or DAPI)/CFP/YFP and DsRed (or Texas Red)

| exciter | S375/20 |
|----------------------|---------|
| exciter | S436/10 |
| exciter | S492/18 |
| exciter | S580/20 |
| 86010bs beamsplitter | |
| emitter | S415/24 |
| emitter | S465/25 |
| emitter | S530/40 |
| emitter | S630/60 |
| | |

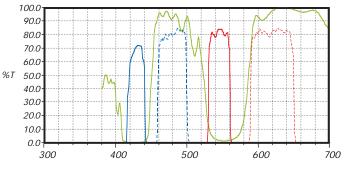
These filter sets usually require a filter wheel on excitation and emission. The beamsplitter remains stationary while the excitation and emission filters change. The proteins are individually excited and their emissions are captured sequentially.



Used for the sequential imaging of Blue, Cyan and Yellow GFPs and DsRed. CFP excitation specifically designed for use with mercury excitation.

86011 for CFP and DsRed

| exciter | S430/25 |
|----------------|--------------|
| exciter | S545/30 |
| 86011bs | beamsplitter |
| emitter | S480/40 |
| emitter | S620/60 |
| | |



Wavelength (nm)

Used for the sequential imaging of Cyan GFP and DsRed. Can be used with both mercury and xenon excitation.



REFERENCES:

Cover Image: Fluorescent Protein Spectra by George Patterson, Rich N. Day and David Piston, Courtesy *Journal of Cell Science* 2001 (114, pp. 837-838).

Inside Cover Image: Dr. Steve Kay and Dr. Jeff Plautz, University of Virginia.

Green Fluorescent Protein: Properties, Applications, and Protocols. Edited by Martin Chalfie and Steven Kain. Wiley-Liss, 1998.

Green Fluorescent Proteins: Methods in Cell Biology, Vol. 58. Edited by Kevin F. Sullivan and Steve A. Kay. Academic Press, under the auspices of the American Society for Cell Biology, 1999.

Cells: A Laboratory Manual. David L. Spector, Robert D. Goldman and Leslie A. Leinwand. Cold Spring Harbor Laboratory Press, 1998.

Fluorescence Microscopy. Microscopy Handbooks 40, Second Edition. Brian Herman. Springer-Verlag, in association with the Royal Microscopical Society, 1998.

A very special thanks to all the researchers who have given their continued comments, support or recommendations. There are far too many of these special professionals to list here, but they know who they are.

© Copyright 2001 Chroma Technology Corp.









74 Cotton Mill Hill, Unit A-9 Brattleboro, VT 05301 USA 800.824.7662 (800-8 CHROMA) 802.257.1800 Fax 802.257.9400 e-mail: gfp@chroma.com www.chroma.com