Applications for Molecular Imager FX™ Systems: Instrument Settings

Table 1. Filter selection by application.

Application	Laser	Filter* Emission Filter Wheel A	Filter* Emission Filter Wheel B	Filter* Excitation Filter Wheel C**
Radioisotopes				
CS or BI screen (Bio-Rad)**	1,064 nm**	Blank (1)**	IR blocking**	532 nm blocking (1)**
K screen (Kodak)	532 nm	Blank (1)	532 and NIR block; 390 nm BP (2)**	1,064 nm blocking (2)**
Fuji screen	532 nm	Blank (1)	532 and NIR block; 390 nm BP (2)**	1,064 nm blocking (2)**
Chemifluorescence				
ECL Plus	488 nm	530 nm BP (2)	Blank (4)	Blank (4)
AttoPhos	488 nm	530 nm BP (2)	Blank (4)	Blank (4)
Chemiluminescence				
CH screen	1,064 nm**	Blank (1)	IR blocking (2)**	532 nm blocking (1)**
DNA-stained gel				
Ethidium bromide	532 nm	Blank (1)	555 nm LP (3)	1,064 nm blocking (2)**
SYBR Green I and II	488 nm	530 nm BP (2)	Blank (4)	Blank (4)
SYBR Gold	488 nm	530 nm BP (2)	Blank (4)	Blank (4)
Protein-stained gel				
SYPRO Orange	488 nm	530 nm BP (2)	Blank (4)	Blank (4)
SYPRO Red	532 nm	Blank (1)	555 nm LP (3)	1,064 nm blocking (2)**
Nile Red	532 nm	Blank (1)	555 nm LP (3)	1,064 nm blocking (2)**
SYPRO Ruby	532 nm	Blank (1)	555 nm LP (3)	1,064 nm blocking (2)**
Deep Purple	532 nm	Blank (1)	555 nm LP (3)	1,064 nm blocking (2)**
Fluorophores				
Alexa Fluor 488	488 nm	530 nm BP (2)	Blank (4)	Blank (4)
Alexa Fluor 532	532 nm	Blank (1)	555 nm LP (3)	1,064 nm blocking (2)**
Alexa Fluor 546	532 nm	Blank (1)	555 nm LP (3)	1,064 nm blocking (2)**
Alexa Fluor 635	635 nm	690 nm BP (4)	Blank (4)	Blank (4)
FITC	488 nm	530 nm BP (2)	Blank (4)	Blank (4)
FAM	488 nm	530 nm BP (2)	Blank (4)	Blank (4)
Cy2	488 nm	530 nm BP (2)	Blank (4)	Blank (4)
Cy3	532 nm	Blank (1)	555 nm LP (3)	1,064 nm blocking (2)**
Cy5	635 nm	690 nm BP (4)	Blank (4)	Blank (4)
HEX	532 nm	Blank (1)	555 nm LP (3)	1,064 nm blocking (2)**
R6G	532 nm	Blank (1)	555 nm LP (3)	1,064 nm blocking (2)**
TAMRA	532 nm	Blank (1)	555 nm LP (3)	1,064 nm blocking (2)
Texas Red	532 nm	640 BP (3)	Blank (4)	1,064 nm blocking (2)**
Microplate				
DNA (SYBR Green I)	488 nm	530 nm BP (2)	Blank (4)	Blank (4)
Protein (NanoOrange)	488 nm	530 nm BP (2)	Blank (4)	Blank (4)
ssDNA (OliGreen)	488 nm	530 nm BP (2)	Blank (4)	Blank (4)
DNA (PicoGreen)	488 nm	530 nm BP (2)	Blank (4)	Blank (4)
β-Galactosidase (fluorescein di-β-D-galactopyranoside)	488 nm	530 nm BP (2)	Blank (4)	Blank (4)
Multiplexing				
DIGE (Cy2)	488 nm	530 nm BP (2)	Blank (4)	Blank (4)
DIGE (Cy3)	532 nm	Blank (1)	555 nm LP (3)	1,064 nm blocking (2)**
DIGE (Cy5)	635 nm	690 nm BP (4)	Blank (4)	Blank (4)
SYPRO Ruby	532 nm	Blank (1)	555 nm LP (3)	1,064 nm blocking (2)**
Pro-Q Diamond	532 nm	Blank (1)	555 nm LP (3)	1,064 nm blocking (2)**
Pro-Q Emerald	488 nm	530 nm BP (2)	Blank (4)	Blank (4)
Densitometry				
Coomassie Blue gel/blot	532 nm	Blank (1)	555 nm LP (3)	1,064 nm blocking (2)**
Copper stained gel/blot	532 nm	Blank (1)	555 nm LP (3)	1,064 nm blocking (2)**
Silver stained gel/blot	532 nm	Blank (1)	555 nm LP (3)	1,064 nm blocking (2)**
X-ray film (gray type)	532 nm	Blank (1)	555 nm LP (3)	1,064 nm blocking (2)**

* Numbers in parentheses define filter positions on the filter wheels.

** FX model and accessories for it are discontinued and no longer available. Current models are FX Pro and FX Pro Plus.

BIO-RAD

Important Guidelines for Molecular Imager FX Systems

Loading the Gels Into Molecular Imager FX Systems

Bio-Rad's Ready Gel®, Criterion™, and PROTEAN® II precast gels must be removed from their cassettes and placed on the standard glass sample tray. Gels in glass cassettes may be scanned while in the cassettes as long as the glass has low fluorescence. Removing at least the top glass plate prior to scanning is recommended. Scans obtained through top glass plates are at least 20-fold less intense than those obtained with the plates removed. Gels removed from their cassettes should be placed on the standard glass sample tray. For gels between thick glass plates, the multi-sample tray II should be used with an appropriate spacer to bring the sample into the proper focal plane for imaging.

Instrument Settings

All settings of the FX instruments are performed through the controls in the acquisition modules of Quantity One® and PDQuest™ software. The FX acquisition screen contains preset applications to automatically select the correct laser and filter combinations.

Emission Filters

How to Determine Filter Positions

It is important that the filters be installed in their proper positions on the filter wheels, as indicated in Table 1 (position numbers in parentheses).

To find out what filter is in the light path, check the configuration of the filter wheels A and B located at the front panel. Filter wheel C is present on the FX model only and does not exist on newer Molecular Imager FX™ Pro and Molecular Imager FX™ Pro Plus instruments. It is located on the right side of the front panel and protected by a cover that can be removed for observation of the filters.

The cover should be removed from the front panel to expose the filter holders. There are four filter holders on both filter wheels A and B. If the exposed filter holder displays number 1, it means that filter number 3 (opposite location on the filter wheel) is in the light path and ready to be used for imaging, as shown in Figure 1. Similarly, if you see a filter holder displaying number 2, it means that filter holder 4 is in the light path.

Fig. 1. The filter wheels can be viewed when cover is removed.

How to Identify Filters

- The filters for the Molecular Imager FX systems are of three types: band pass (BP), long pass (LP), and blocking
- With purchase of the optional 635 nm external laser, the 555 nm LP filter is replaced with a 605 nm BP filter to distinguish Cy5 from 532 nm excitable dyes

An OptiGreen filter is used for detection of Cy2 and FITC with 532 nm excitation. Use a custom photomultiplier tube (PMT) voltage setting of 60–100% for OptiGreen. For the detection of Cy5, we recommend using a PMT voltage setting of 65–85%, depending on the concentration of Cy5 in the sample.

Table 2. Filters.

Catalog #	Type	Description
170-7896	BP	640DF35
170-7864	Blocking	532 nm and NIR block
170-7895	BP	530DF28
170-7867	—	Blank filter holder
170-7866	BP	605DF50
170-7865	BP	695RDF55 reflect 635 nm
170-7863	LP	555LP reflect 532 nm
170-7868	BP	HQ505/20

Optimizing Imaging

We recommend that you do a quick initial scan at 800 µm resolution to find the location of your sample and determine its approximate intensity. Select the Highlight Saturated Pixels box to aid in interpreting sample intensity. No more than a few of the brightest spots should contain saturated pixels. If an image contains too many saturated pixels, reduce the PMT voltage.

Imaging Samples With Different Intensities

Sample intensity for fluorescent applications reflects PMT voltage settings. If a sample intensity is low, select Low as the detection setting in the Quantity One or PDQuest acquisition modules. It will correspond to a PMT voltage setting of 55%. Medium sets the PMT at 45%, and High sets it at 35%.

To adjust the PMT voltage, you may use the Custom application (Select/Custom/Create) in the software (Figure 2).

Fig. 2. Fine adjustment of PMT voltage.

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