



Hints

- Build panels starting with the targets that provide the fewest formats available.
- Reserve brighter fluorochromes for antigens expressed at low levels. (e.g., Use PE or APC for intracellular cytokines.)
- Cross-beam compensation can be difficult to reconcile. Consider a different fluorochrome, or assign any fluorochrome that can be excited by multiple lasers to all possible detection channels.
- Use the Antibody Panel Development Wizard as an exploratory tool to observe the effect of different instrument configurations or to see how the addition of different fluorescent proteins will affect the development of the panel.
- Save your panels for later refinement and reuse.
- Load and edit saved panels from other user via the Fluorish.com website.
- The Fluorish website contains the full online catalogs for all partner vendors, provides the ability to submit and share antibody panels with the online community, and offers a number of panels used in published data and a commentary section for all antibodies.

Links and References

Fluorish LLC. (www.fluorish.com <<http://www.fluorish.com>>)

Shapiro HM. *Practical flow cytometry*. Fourth edition. New York: Wiley-Liss; 2003.



FlowJo is a product of Tree Star, Inc.
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TN.PanelWizard.20100420

Abstract

The **Antibody Panel Wizard** provides a simple interface for designing complex experimental panels. It presents a stepwise process of instrument selection and configuration, and target-fluorochrome refinement, using the combined product databases from multiple major manufacturers. The Wizard will provide substantial time savings, and better panel design than manually searching catalogs, checking fluorescence excitation/emission profiles, and matching target-fluorochrome combinations to the appropriate laser/detector of the instrument. The Wizard reduces compensation issues, including cross-beam compensation, by automatically assigning fluorochromes to specific detectors based on their excitation and emissions profiles. Default instrument settings are provided, or you can easily add your custom configuration to the wizard. With links to the Fluorish e-commerce site, antibodies can now be ordered from multiple vendors at once.

General Information

FlowJo's Antibody Panel Wizard is designed to create an efficient and effective way of determining flow cytometry reagent requirements. As the complexity of experimental panels increase, there are more spectral overlaps between fluorochromes and dyes, and proper assignment of conjugates is the key to improving the efficiency of the assay.

The Wizard is a novel approach to antibody panel creation. It provides a number of restriction steps in order to refine the generated searches for reagents. These steps sequentially define the constraints on your experiment, asking you to: select your cytometer from a comprehensive list; specify the instrument's laser, filter and detector configuration; choose any non-antibody-bound fluorochromes used in the panel, and search options for antibody-bound fluorochromes and antibody targets, based on species specification. As a result of the selections made in these steps, a list of product results is provided from several manufacturers specific to the target-fluorochrome. A large number of formats per target are listed in the results panel for an immediate indication of possible

starting points. As target-fluorochrome antibodies are selected for the panel, they are assigned to the optimal laser/detector combination of the specified instrument configuration based on excitation efficiency and peak emission.

After each assignment, the Wizard refines the results to remove fluorochromes with similar emission profiles. This refinement process provides for the quick development of antibody panels, substantially reducing the user error of possibly selecting fluorochromes with significant overlapping emissions. The final step of the Wizard will prompt you to save, email, or print the panel. You will have the option of ordering the reagents selected directly from the panel, even when products from multiple vendors have been selected. Users will also be encouraged to share their panels online in a Fluorish public forum, allowing others the convenience of utilizing panels that have already been created, and making it easier to reproduce results.

This technical note provides excerpts of selected panels in the Panel Wizard with instructions.

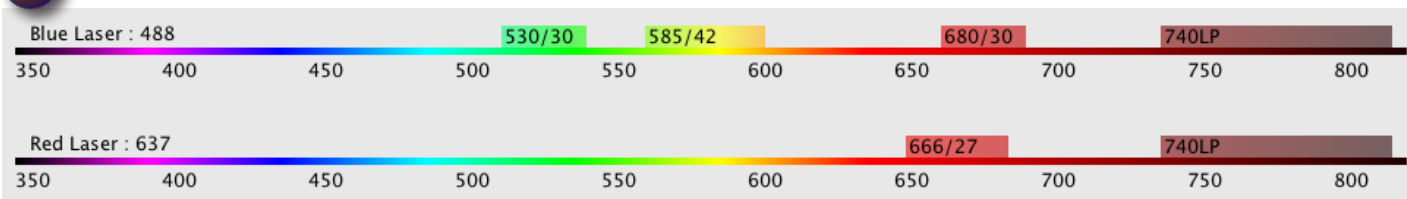


Panel Wizard

1 Select your cytometer from the list of all instruments.

Save the cytometer configuration from BD's CS&T software and drop that file onto the panel wizard. It will adjust the cytometer configuration to match the filter and detectors in the instrument.

2 Customize the configuration to specify the lasers and detectors in your lab.



3 Select Dyes and Fluorochromes to use in the experiment.

Dyes and Fluorochromes are both annotated with their peak excitation frequency. Selecting items will color other items that have spectral overlap with the current list. Dyes precede conjugated fluorophores in the assignment to panels.

4 Select the target proteins used in your experiment. Proteins are organized by species. Build the list to use in the panel by selecting from the left list.

5 Match the targets and fluorochromes into conjugations.

There is a variety of conjugates available for many targets, and fewer for others.

CatalogNu...	Vendor	Target	Fluorochro...	Clone	Isotype	Species	Amount	Price
302011	Biolegend	CD16	APC	3G8	Mouse IgG1;...	Human; Chimpanzee; Bab...	25 tests	120
302012	Biolegend	CD16	APC	3G8	Mouse IgG1;...	Human; Chimpanzee; Bab...	100 tests	250
555415	BD Biosciences	CD19	APC	HIB19	IgG1; k	Human	100 tests	-
302211	Biolegend	CD19	APC	HIB19	Mouse IgG1;...	Human; Chimpanzee; Bab...	25 tests	105
302212	Biolegend	CD19	APC	HIB19	Mouse IgG1;...	Human; Chimpanzee; Bab...	100 tests	230
555415	BD Biosciences	CD19	APC	HIB19	IgG1; k	Human	100 tests	-
302211	Biolegend	CD19	APC	HIB19	Mouse IgG1;...	Human; Chimpanzee; Bab...	25 tests	105
302212	Biolegend	CD19	APC	HIB19	Mouse IgG1;...	Human; Chimpanzee; Bab...	100 tests	230
559776	BD Biosciences	CD20	APC	2H7	IgG2b; k	Human	100 tests	-
302309	Biolegend	CD20	APC	2H7	Mouse IgG2...	Human; Chimpanzee; Bab...	25 tests	120
302310	Biolegend	CD20	APC	2H7	Mouse IgG2...	Human; Chimpanzee; Bab...	100 tests	250
555335	BD Biosciences	CD3	APC	UCHT1	IgG1; k	Human	100 tests	-
555342	BD Biosciences	CD3	APC	HIT3a	IgG2a; k	Human	100 tests	-
340440	BD Biosciences	CD3	APC	SK7	IgG1; k	Human	100 tests	-
300311	Biolegend	CD3	APC	HIT3a	Mouse IgG2...	Human	25 tests	115
300312	Biolegend	CD3	APC	HIT3a	Mouse IgG2...	Human	100 tests	245
300411	Biolegend	CD3	APC	UCHT1	Mouse IgG1;...	Human; Chimpanzee	25 tests	115
300412	Biolegend	CD3	APC	UCHT1	Mouse IgG1;...	Human; Chimpanzee	100 tests	245
317317	Biolegend	CD3	APC	OKT3	Mouse IgG2...	Human	25 tests	115
317318	Biolegend	CD3	APC	OKT3	Mouse IgG2...	Human	100 tests	250

The catalogs of several vendors are aggregated into a single database to enable you to search across all companies to build your panel. Experiments will no longer be limited by the logistics of searching and purchasing reagents.