Panel Wizard

FLOWJO
Flow Cytometry
Analysis Software

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 of target-fluorochrome refinement, using combined product databases from multiple major manufacturers. Using 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. Because the Wizard includes 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 increases, 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. Based on the selections made in these steps, a list of product results is provided from several manufacturers. 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 thus making it easier to reproduce results.

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

Panel Wizard

1 Select your cytometer from the list of all instruments.

BD Biosciences | Beckman Coulter | Cytek | Millipore | Partec | Accuri | Other

- FACScan
- FACSCalibur 1 Laser
- FACSCalibur 2 Laser
- FACSCanto
- FACSCantoII
- FACSVantage
- FACSArray
- LSRI
- LSRII
- FACSAria
- FACSAriaII
- FACSAriaIII
- inFlux
- LSRFortessa

- EPICS XL 3
- EPICS XL 4
- EPICS Elite
- FC500
- MoFlo
- Gallios
- Cyan ADP 7 color
- Cyan ADP 9 color

- xP4
- xP5
- DxP6
- DxP8
- FacsScan
- FacsCalibur
- FacsSort
- FacsVantage

- easyCyte 5HT
- easyCyte 6HT
- easyCyte 6HT/2L
- easyCyte 8HT
- easyCyte Mini
- PCA 96
- PCA

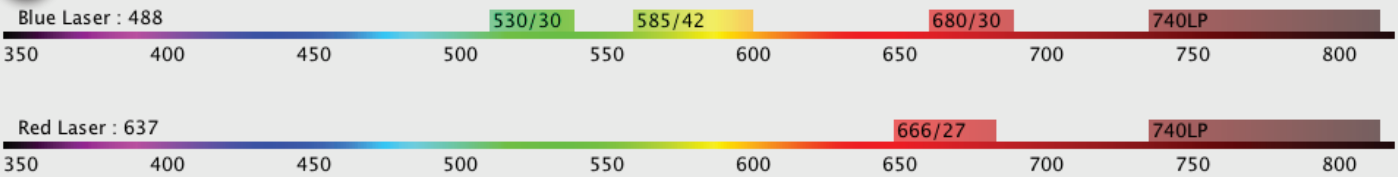
- CyFlow ML
- CyFlow space
- CyFlow SL

- C6

- Miltenyi MACSQuant
- Amnis ImageStream
- iCyt Reflection
- Stratadigm S1000

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.

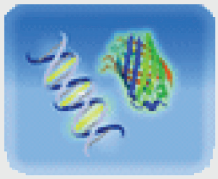
2 Customize the configuration to specify the lasers and detectors in your lab.



3 Select Dyes and Fluorochromes to use in the experiment.

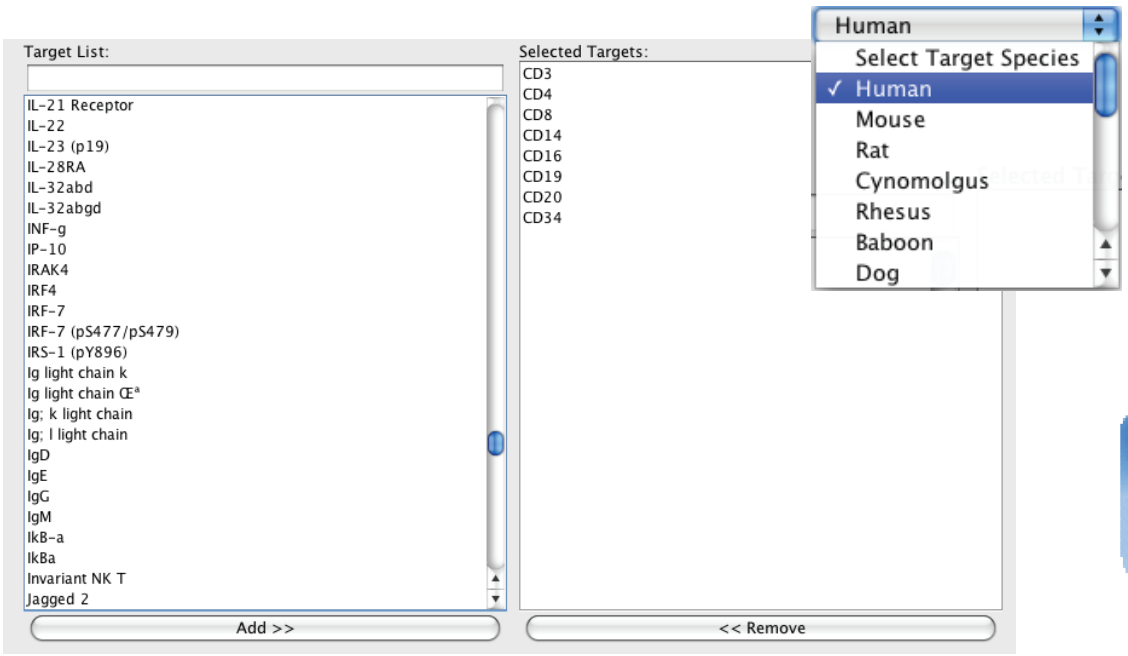
Dyes and Fluorochromes are both annotated with their peak excitation frequency. Selecting items will color other items that have spectral overlap with the current list. Dyes precede conjugated fluorophores in the assignment to panels.

- Marina Blue(461)
- DyeCycle Violet(437)
- Calcein Violet AM(452)
- RiboFlavin(531)
- GFP(515)
- CFSE(525)
- Fluo-4(516)
- ZsGreen(517)
- Fluoro-Emerald(523)
- Calcein(517)
- Rho 110(520)
- SYBR Green(522)
- Thiazole Orange(530)
- PicoGreen(522)
- CyQUANT DNA(522)
- Acridine Orange(525)
- SYTOX Green(523)
- DCFH(525)
- YFP(530)
- DyeCycle Green(533)
- Fluo-3(526)
- Magnesium Green(531)
- Rho 123(529)
- TO-PRO-1(533)
- TOTO-1(533)
- EYFP(528)
- mCitrine(529)
- Fura Red Lo(672)
- 7-AAD(647)
- Nile Red(637)
- SNARF-1 pH9(640)
- mPlum(649)
- Phycocyanin(646)
- PerCP-Cy5.5(695)
- PerCP(675)
- DyLight 488(518)
- Alexa Fluor 488(519)
- FITC(520)
- PE(578)
- PE-Cy5.5(695)
- Cy5(670)
- Alexa Fluor 647(665)
- APC(660)



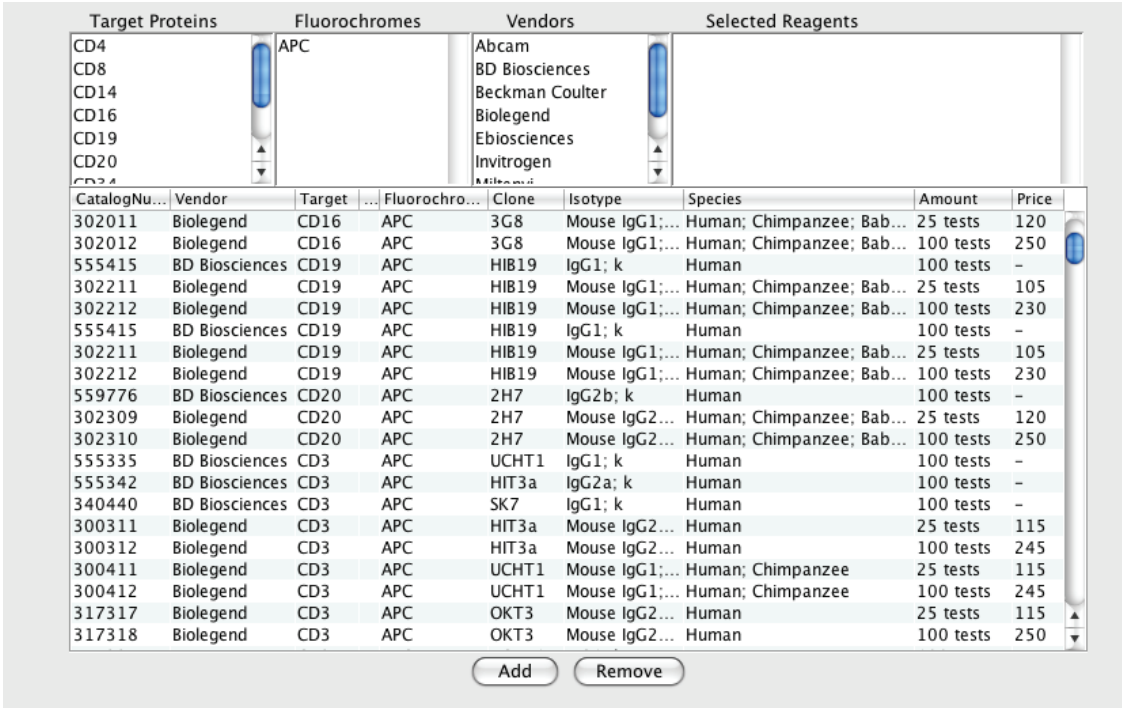
4 Select the target proteins used in your experiment.

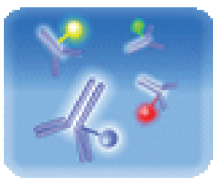
Proteins are organized by species.
Build the list to use in the panel by selecting from the left column.



5 Match the targets and fluorochromes into conjugations.

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





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.

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 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 to explore 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 users via the Fluorish.com website.
- The Fluorish.com website contains the full online catalogs for all partner vendors, provides the ability to submit and share antibody panels with the online community, offers a number of panels used in published data, and includes a commentary section for all antibodies.

TREE STAR

FlowJo is a product of Tree Star, Inc.
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