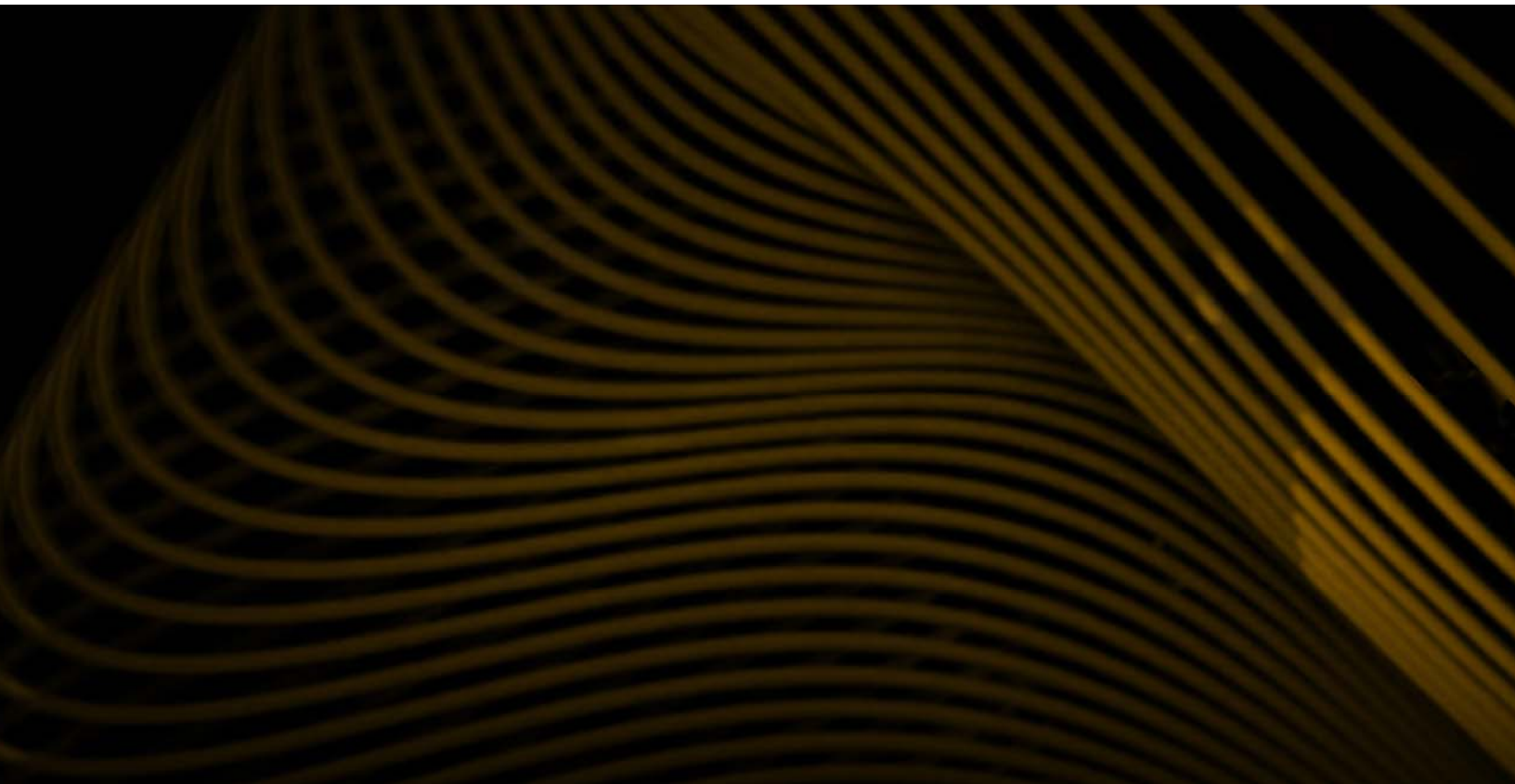


# Maxpar Panel Designer

USER GUIDE



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# Maxpar Panel Designer

## Introduction

Multiparametric cytometry analysis platforms have sufficient sensitivity, dynamic range and resolution to independently measure a wide number of targets simultaneously. In mass cytometry these targets, such as cell surface markers, signaling proteins, cytokines, and nucleic acids are identified with target-specific antibodies conjugated to rare earth metals, and the array of metal-conjugated antibodies (MCAs) used to define a system is referred to as a panel.

When designing a panel, it is important to develop a strategy that provides maximum signal detection with minimum background in the channels for protein targets that are difficult to detect. Signal overlap is a common form of background in which signal derived from a probe is measured in undesired channels. One of the chief advantages of mass cytometry is that signal overlap is small and measureable.

The Maxpar<sup>®</sup> Panel Designer is an interactive web-based application that simplifies and optimizes panel design. It allows users to build a panel consisting of MCAs from the Fluidigm<sup>®</sup> catalog and from custom conjugates. The tool calculates and provides visualization of predicted signal overlap for each panel, and also has an algorithm that optimizes the metal tag selections for each target to optimize panel performance. Panels can be saved and exported for sharing with collaborators and for future import into CyTOF<sup>®</sup> instrument control software.

## Importance of Signal and Background in Panel Design

In order to quantify the amount of each targeted protein, the antibody panel must provide sufficient sensitivity for each protein targeted by the panel. Sensitivity is the ratio of signal derived from the target to background derived from undesired sources. Therefore, optimal panel design provides sufficient signal to resolve its protein targets over any background present in the system.

## Signal

Target-specific signal detected in its mass channel depends on the abundance of the target, performance of the probe, and the instrument's detection efficiency for the metal isotope tag.

## Isotope Detection

The CyTOF instrument employs a detector capable of simultaneously detecting over 90 isotopes separated by their time-of-flight (TOF). Each isotope delivers a similar signal to the detector. However, the ion optics within the CyTOF instrument are tuned for optimal delivery of metals in the 153–176 Da range to the detector. For example, the system delivers to the detector approximately three times as much  $Tb^{159}$  as the lowest mass in the lanthanide series ( $^{139}La$ ) and 1.5 times as much  $Tb^{159}$  as the heaviest lanthanide isotope ( $^{176}Yb$ ) (Figure 1).

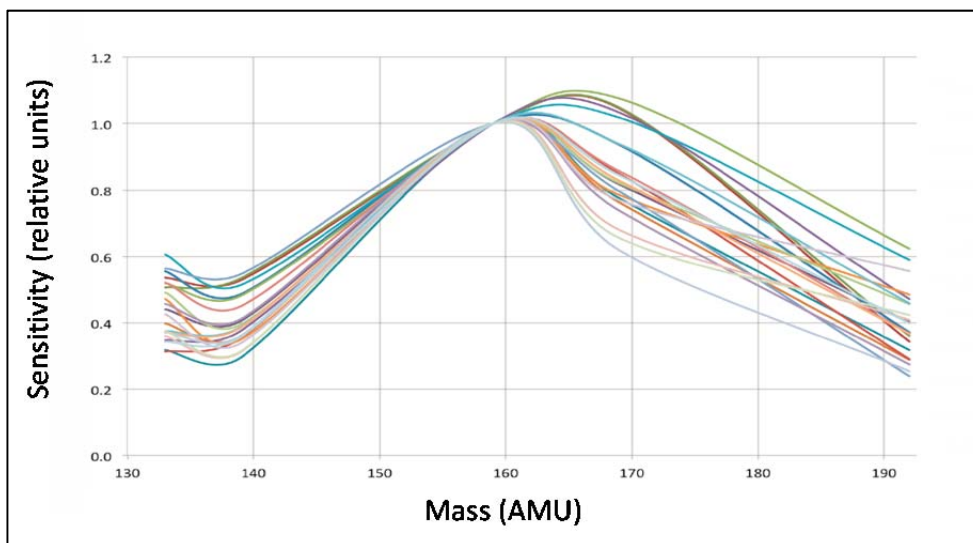


Figure 1: Mass response curve

Relative counts (y-axis) detected in each mass channel (x-axis) for 24 CyTOF instruments. The relative count measured in a given channel is directly proportional to the transmission efficiency of the corresponding isotope through the ion optics of the mass cytometer. The mass range shown covers all isotopes in the lanthanide series of elements.

## Probe Performance

Metal-conjugated antibody (MCA) probes are created by conjugating metal-loaded polymers (mass tags) to antibodies under partial reducing conditions. Probes that deliver optimal signal have not only a high metal content but also retain high avidity for the antigen. Because of this, it is very important to empirically determine the signal for each probe by titrating the reagent on cells that will be used in the experimental system.

## Background

Background refers to undesired signal in a target's measurement channel. In mass cytometry the main source of background is signal overlap from metal tags into other channels. Signal overlap in mass cytometry is small and measurable, and includes overlap due to isotope purity, oxide formation, and abundance sensitivity (Figure 2). These are characterized by a matrix that quantifies percent overlap of all metal tags into all channels, and this matrix is utilized by the Maxpar Panel Designer to quantify predicted background signal in all channels in the panel (Figure 3).

## Abundance Sensitivity

In time-of-flight mass spectrometry, ions are resolved according to their relative velocities, which are determined by their mass and kinetic energy. In the incoming beam, ions of the same kind have small differences in initial position and velocity from each other, and these position and velocity spreads lead to broadening of the apparent mass peak resulting in some signal measurable in adjacent mass channels. Abundance sensitivity is defined as the ratio of the signal observed at the  $M\pm 1$  mass channel to the signal at mass  $M$ . It is a fixed value for a specific instrument under specified operating conditions (typically less than 2% for CyTOF and less than 0.3% for CyTOF 2 and Helios instruments).

## Oxides

Some ions form strong oxides that survive at low levels in the ionizing plasma and appear in the  $M+16$  channels (Figure 2,  $M+16$  peak). The abundance of oxides at the high temperature of the plasma ionization source is determined by the atomic ion-oxygen bond strength. Thus, La, Ce, Pr and Nd isotopes produce the largest  $M+16$  signal (typically 2%–3%), while the Eu isotopes form the weakest bonds (typically  $<0.1\%$   $M+16$  signal). Reducing the level of oxides to a minimum is dependent on optimizing the plasma temperature, which is part of the normal tuning procedure.

## Isotopic Purity

Naturally occurring elements are a mixture of isotopes which can be commercially obtained in enriched form. The enrichment that is possible for a candidate mass tag is related to the natural abundance of the isotopes of that element, and when the isotope is naturally greater than 20% abundant, an enrichment of better than 98% is often achievable. The purity of each isotope is determined at the time of its production.

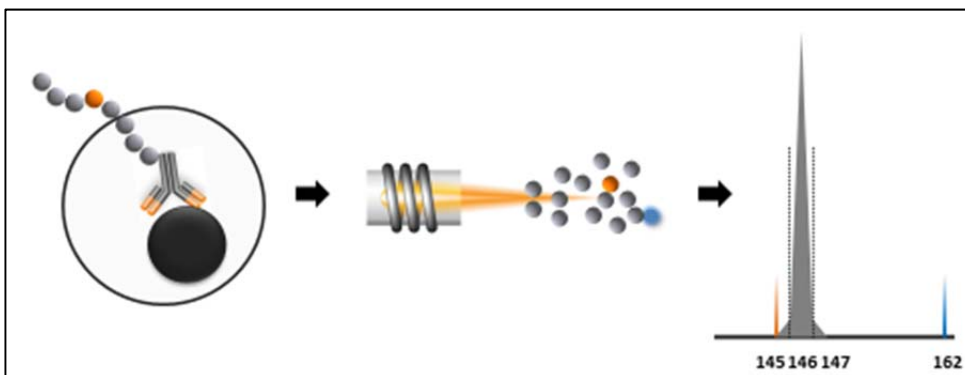


Figure 2: Three types of signal overlap in mass cytometry

A cell bound by an antibody conjugated to  $^{146}\text{Nd}$  (gray circles) that contains trace  $^{145}\text{Nd}$  (orange circles) is shown at left. The cell is ionized in the plasma (center). Some  $^{146}\text{Nd}$  ions form oxides in the plasma (blue). Ions are separated by mass in the TOF chamber and strike the detector in time-resolved channels (right). Mass graph illustrating the signal overlap from oxides appearing in the M+16 channel (blue peak), from abundance sensitivity in the M $\pm$ 1 channels (gray shoulders), and from  $^{145}\text{Nd}$  in the 145 channel (orange peak).



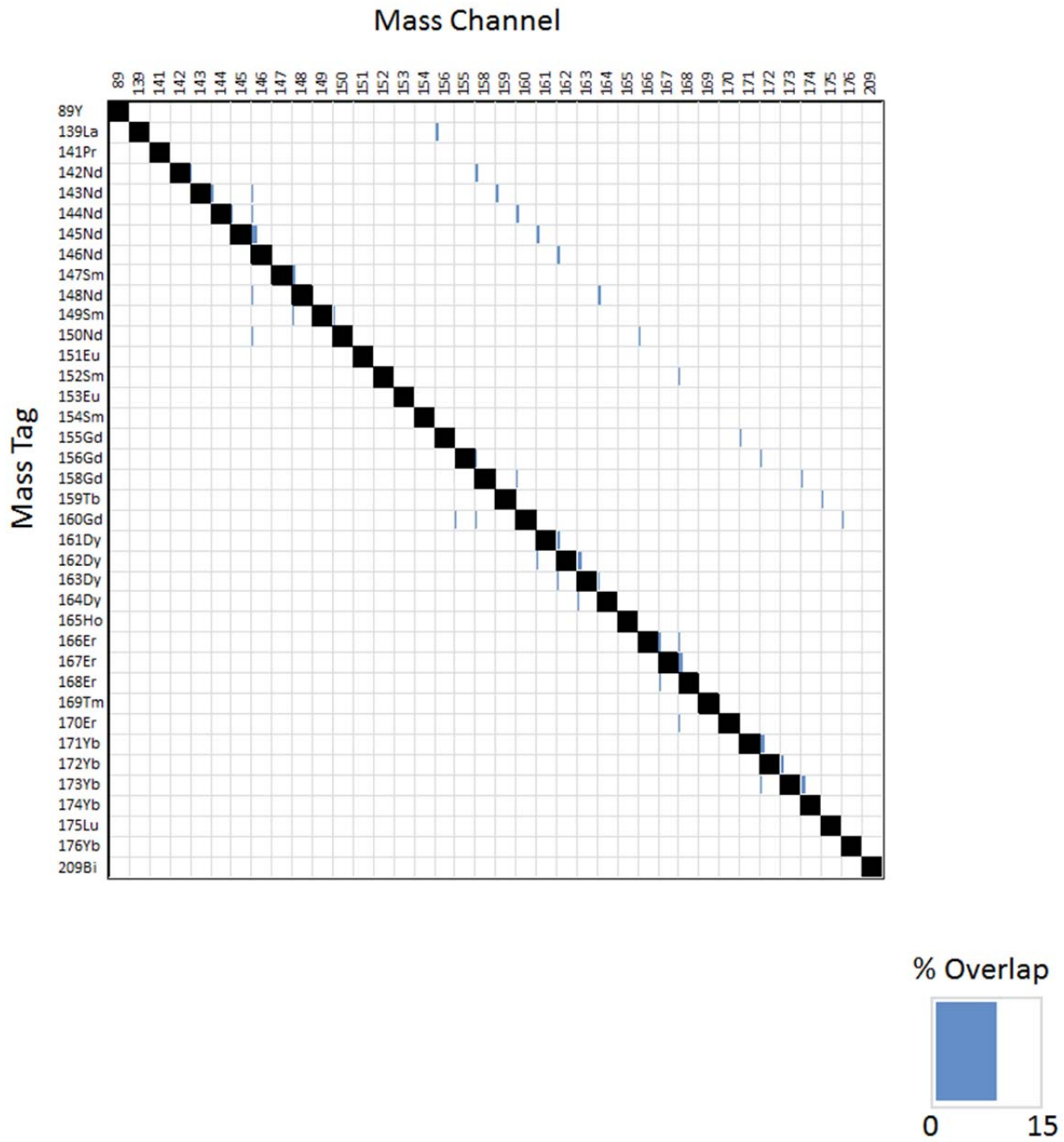


Figure 3: Percent signal overlap matrix

The signal overlap derived from each mass tag (rows) into each channel (columns) is expressed as a percentage (blue bar within each box) of signal in the desired channel.

The Maxpar Panel Designer calculates the background (in counts) derived from signal overlap (SO) expected in all channels of the panel using the following formula:

$$SO = S \times (PO/100)$$

where SO is signal overlap from a donor channel to a recipient channel, S is signal expected in the donor channel, and PO is percent overlap from the donor channel to the recipient.

## Signal and Tolerance Values Used by the Maxpar Panel Designer

Optimal panel design minimizes background in channels used to measure difficult-to-detect targets. In other words, probes should be chosen that place low-signal targets that tolerate the least signal overlap into low background channels, and high-signal targets that can tolerate more signal overlap into higher background channels.

Thus, the signal characteristics of a target dictate not only the location and quantity of background in channels outside its channel, but also the tolerance for signal overlap within its channel. Each target added to a panel within the Panel Designer has a signal and tolerance value, and these values are used along with the percent overlap matrix by the Panel Designer to guide optimal panel design.

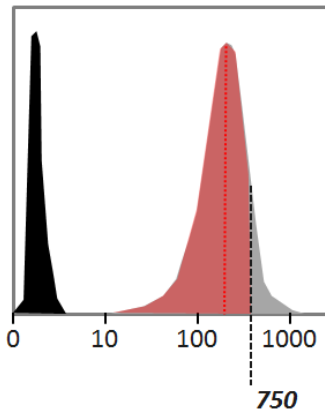
Signal is the expected 75<sup>th</sup> percentile dual count value of the population expressing the highest amount of a given target. This value is multiplied by the PO matrix to predict the external channel background derived from signal overlap.

The tolerance value is 20% of the 75<sup>th</sup> percentile for the population expressing the lowest amount of a given target:

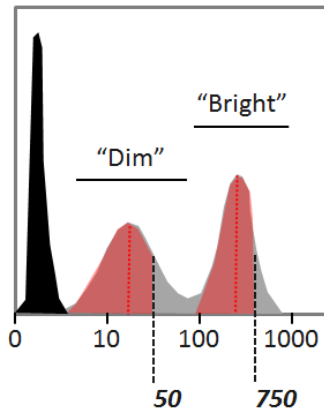
$$T = 0.2 \times S_{75L}$$

This value is compared to the cumulative signal overlap within the channel to gauge the appropriateness of the channel for the target. For a unimodal population, tolerance is simply 20% of the signal value (Figure 4, example 1). For a multimodal population, however, tolerance can be much less than 20% of the signal value (Figure 4, example 2). This is desired since the bright population contributes the dominant signal overlap, but the dim population tolerates less background and therefore dictates the tolerance value.

**Example 1:**  
Unimodal distribution of the signal



**Example 2:**  
Bimodal distribution of the signal



**Example 3:**  
Inducible signal

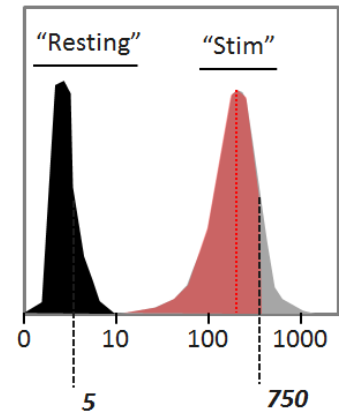


Figure 4: Signal and tolerance  
In dual counts (DC) for the three example distributions.

For inducible antigens that increase or decrease with treatment, the range of expression is considered for the tolerance value according to the following formula:

$$T = 0.2 \times [S_{75L} + 0.1 \times (S_{75H} - S_{75L})]$$

where  $S_{75L}$  is the 75<sup>th</sup> percentile dual count value for the condition producing the lowest amount of the given target and  $S_{75H}$  is the 75<sup>th</sup> percentile dual count value for the condition producing the highest amount.

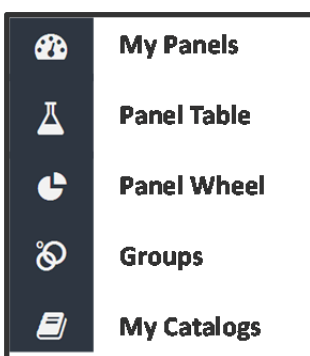
# Maxpar Panel Designer Software

## Accessing the Panel Designer

Log in to the DVS portal with your username and password. Click on the Panel Designer icon. This will take you to the My Panels window.

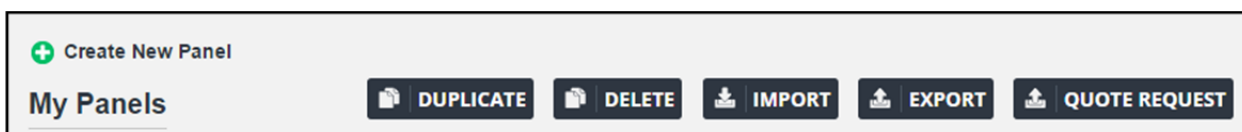
## Toolbar

The left sidebar contains icons that provide click access to each of the five Panel Designer tabs.




## My Panels

This window is used to manage your panels.

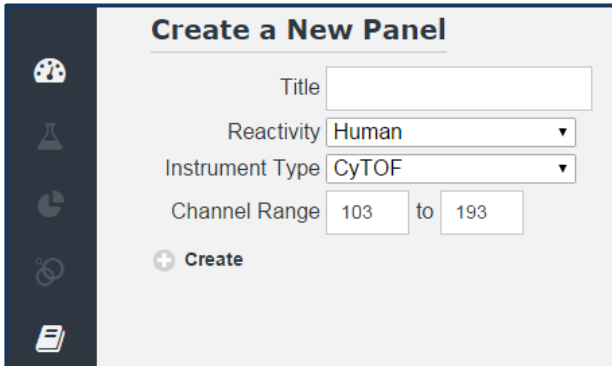


In this window you can:

- 1 Create a new panel by clicking ;
- 2 View or edit an existing panel by clicking its name;
- 3 Duplicate, delete, import, or export a panel, or generate a reagent quote request for a panel, by checking the box next to the panel name and clicking the corresponding button.

## My Panels: Creating a New Panel

Click  to begin a new panel.




**Create a New Panel**

Title

Reactivity

Instrument Type

Channel Range  to


 **Create**

Give the panel a unique name by typing in the Title field.

Select target species from the Reactivity dropdown. This dictates which catalog antibodies are available for the panel.

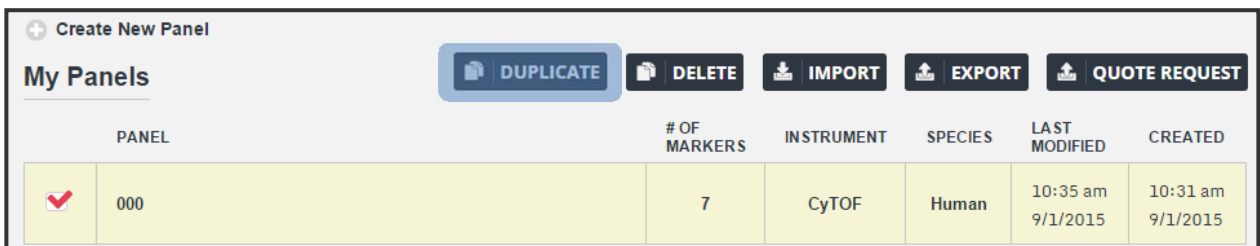
Select instrument from the Instrument Type dropdown. This dictates the percent overlap matrix that will be used since the instruments have different abundance sensitivities.


The default Channel Range corresponding to the selected instrument will appear. This range determines which tags are available for the panel.

Click  to create the panel. The panel will now be visible in the list.





## My Panels: Duplicate Panel

Duplicating allows you to edit a panel while retaining the original. To duplicate, check the box next to the original panel, then click **Duplicate**.



 Create New Panel

**My Panels**

 **DUPLICATE**  **DELETE**  **IMPORT**  **EXPORT**  **QUOTE REQUEST**

	PANEL	# OF MARKERS	INSTRUMENT	SPECIES	LAST MODIFIED	CREATED
<input checked="" type="checkbox"/>	000	7	CyTOF	Human	10:35 am 9/1/2015	10:31 am 9/1/2015

The new panel will appear in the list below the original.

Create New Panel

**My Panels** **DUPLICATE** **DELETE** **IMPORT** **EXPORT** **QUOTE REQUEST**

	PANEL	# OF MARKERS	INSTRUMENT	SPECIES	LAST MODIFIED	CREATED
<input type="checkbox"/>	000	7	CyTOF	Human	10:35 am 9/1/2015	10:31 am 9/1/2015
<input type="checkbox"/>	000 (duplicate)	7	CyTOF	Human	10:48 am 9/1/2015	10:31 am 9/1/2015

### My Panels: Deleting Panels

To delete a panel, check the box next to the panel name, then click **Delete**.

Create New Panel

**My Panels** **DUPLICATE** **DELETE** **IMPORT** **EXPORT** **QUOTE REQUEST**

	PANEL	# OF MARKERS	INSTRUMENT	SPECIES	LAST MODIFIED	CREATED
<input type="checkbox"/>	000	7	CyTOF	Human	10:35 am 9/1/2015	10:31 am 9/1/2015
<input checked="" type="checkbox"/>	000 (duplicate)	7	CyTOF	Human	10:48 am 9/1/2015	10:31 am 9/1/2015

If you confirm the prompt, the selected panel will disappear from the list.

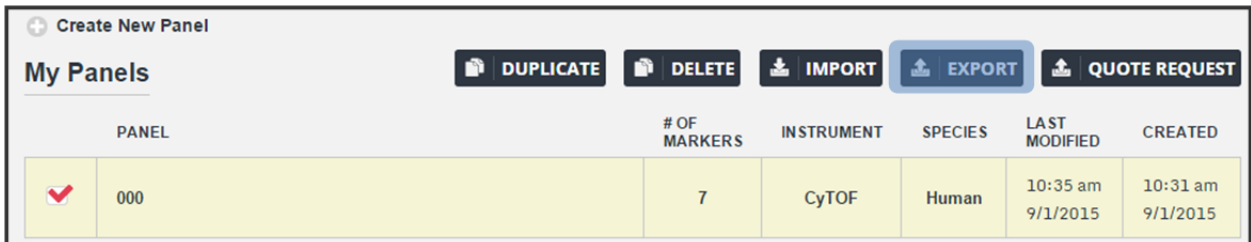
Create New Panel

**My Panels** **DUPLICATE** **DELETE** **IMPORT** **EXPORT** **QUOTE REQUEST**

	PANEL	# OF MARKERS	INSTRUMENT	SPECIES	LAST MODIFIED	CREATED
<input type="checkbox"/>	000	7	CyTOF	Human	10:35 am 9/1/2015	10:31 am 9/1/2015

## My Panels: Sharing Panels Using Import/Export

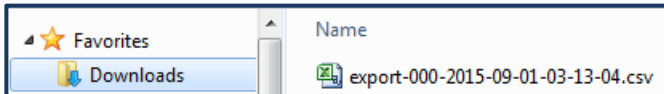
Panels can be exported to a csv file, which can then be imported back into the Panel Designer. This is the method used to share panels with collaborators. To export a panel, check the box next to the panel name, then click **Export**.



The screenshot shows the 'My Panels' interface. At the top, there is a '+ Create New Panel' button. Below it, the title 'My Panels' is followed by five action buttons: 'DUPLICATE', 'DELETE', 'IMPORT', 'EXPORT', and 'QUOTE REQUEST'. The 'EXPORT' button is highlighted with a blue border. Below the buttons is a table with the following columns: 'PANEL', '# OF MARKERS', 'INSTRUMENT', 'SPECIES', 'LAST MODIFIED', and 'CREATED'. There is one row in the table with a checked checkbox in the first column.

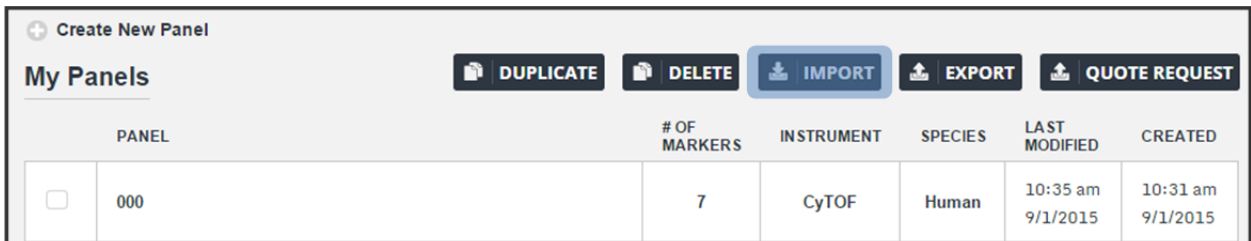
PANEL	# OF MARKERS	INSTRUMENT	SPECIES	LAST MODIFIED	CREATED
<input checked="" type="checkbox"/> 000	7	CyTOF	Human	10:35 am 9/1/2015	10:31 am 9/1/2015

A csv file will be exported to your Downloads folder.



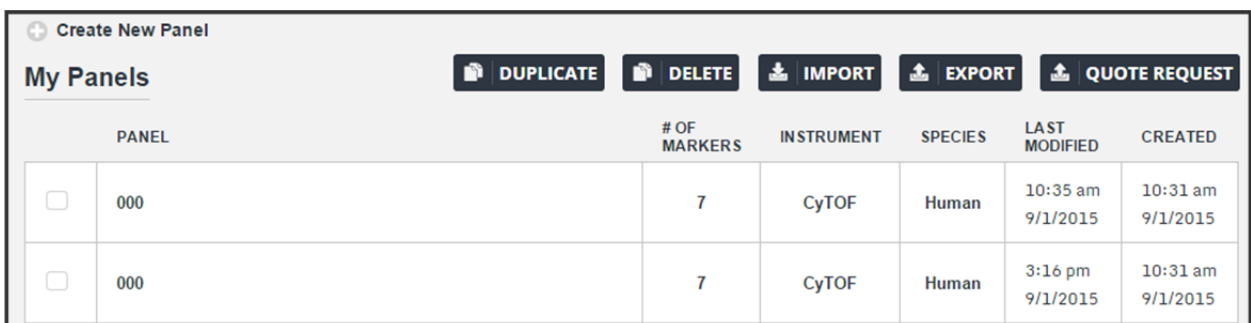
The screenshot shows a Windows file explorer window. The 'Downloads' folder is selected. A single file is visible: 'export-000-2015-09-01-03-13-04.csv'.

To import a panel, click the **Import** button, browse for the panel csv file, then click **Open**.



The screenshot shows the 'My Panels' interface. The 'IMPORT' button is highlighted with a blue border. The table below has one row with an unchecked checkbox.

PANEL	# OF MARKERS	INSTRUMENT	SPECIES	LAST MODIFIED	CREATED
<input type="checkbox"/> 000	7	CyTOF	Human	10:35 am 9/1/2015	10:31 am 9/1/2015



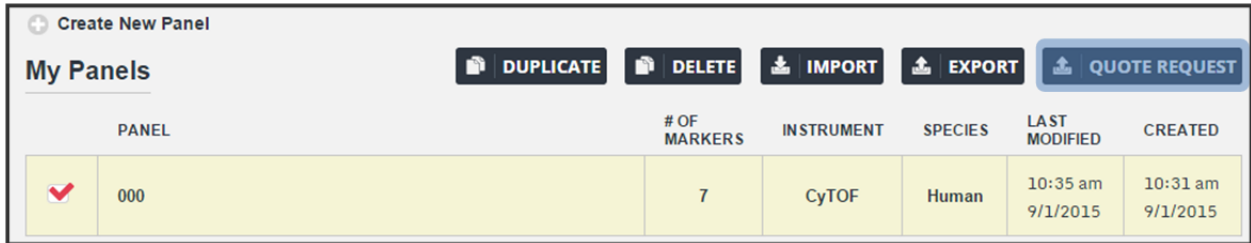
The screenshot shows the 'My Panels' interface. The 'IMPORT' button is highlighted. The table below has two rows, both with unchecked checkboxes.

PANEL	# OF MARKERS	INSTRUMENT	SPECIES	LAST MODIFIED	CREATED
<input type="checkbox"/> 000	7	CyTOF	Human	10:35 am 9/1/2015	10:31 am 9/1/2015
<input type="checkbox"/> 000	7	CyTOF	Human	3:16 pm 9/1/2015	10:31 am 9/1/2015

The imported panel will appear in the list. Note: you can change the name of the imported panel in the Table View (see [‘Panel Table: Renaming the Panel’](#) below).

## My Panels: Generate a Reagent Quote Request

To generate a quote request from a panel, check the box next to the panel name, then click **Quote Request**.



The screenshot shows a user interface for managing panels. At the top left, there is a '+ Create New Panel' button. Below this, the title 'My Panels' is displayed. To the right of the title are five action buttons: 'DUPLICATE', 'DELETE', 'IMPORT', 'EXPORT', and 'QUOTE REQUEST'. The 'QUOTE REQUEST' button is highlighted in blue. Below the buttons is a table with the following columns: 'PANEL', '# OF MARKERS', 'INSTRUMENT', 'SPECIES', 'LAST MODIFIED', and 'CREATED'. The table contains one row with the following data: a red checkmark in the first column, '000' in the second, '7' in the third, 'CyTOF' in the fourth, '10:35 am 9/1/2015' in the fifth, and '10:31 am 9/1/2015' in the sixth.

	PANEL	# OF MARKERS	INSTRUMENT	SPECIES	LAST MODIFIED	CREATED
<input checked="" type="checkbox"/>	000	7	CyTOF	Human	10:35 am 9/1/2015	10:31 am 9/1/2015

This will launch the Quote Request form (see [‘Generating a Quote Request’](#) below). Once the form is completed, a PDF Quote Request will be generated. To receive a quote, email this PDF to [sales@fluidigm.com](mailto:sales@fluidigm.com).



# Panel Table

This window is used to view and edit the selected panel.

CHANNEL	139	140	141	142	143	144	145	146	147	148	149	150	151	152
147Sm	0	0	0	0	0	0	0	3		12.60	1.50	0.60	0	1.20
168Er	0	0	0	0	0	0	0	0	0	0	0	0	0	0
170Er	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	0.00	0.00	0.00	0.00	0.00	0.00	0.00	3.00	0.00	12.60	1.50	0.60	0.00	1.20

In this window you can:

- 1 Rename the selected panel
- 2 Add or remove antibodies to/from your panel
- 3 Add Panel Kits and/or personal panels to your panel
- 4 Choose tags manually or have the Panel Designer assign metals using an algorithm with the Optimize Metals function
- 5 Edit signal and tolerance values for each antibody
- 6 Visualize the predicted signal overlap in all channels used by the panel
- 7 Export the panel to a .csv file
- 8 Generate a quote request for the panel

## Panel Table: Renaming the Panel

To rename the panel, click the pencil icon next to the panel name. Type in the new name and click anywhere outside the panel name box.

## Panel Table: Adding an Antibody to the Panel

Two types of probes can be added to the panel:

- 1 Catalog antibodies: Maxpar preconjugated antibodies from
  - a the Fluidigm catalog
  - b a personal catalog ([see the "My Catalogs" section below](#))

- 2 Custom antibodies: antibodies conjugated using Fluidigm's custom conjugation service or with Maxpar Antibody Labeling kits.

In addition, you can manually choose the tag for the antibody you are adding or leave the tag undecided, in which case the Optimize Metals algorithm will choose the optimal tag.

## Panel Table: Adding a Catalog Antibody

Click **ADD TARGET** to add a new antibody to the panel.

The screenshot shows the Maxpar Panel Designer interface. At the top, there are dropdown menus for Target, Tag, Custom, Signal, and Tolerance. Below these is a blue button labeled "ADD TARGET". Underneath are four dark buttons: "OPTIMIZE METALS", "ADD PANEL", "EXPORT", and "QUOTE REQUEST". On the right side, there is a table with columns labeled "CHANNEL" and values 139, 140, 141, 142, 143. The table contains a row with values 0.00, 0.00, 0.00, 0.00, 0.00.

Type in the target name and select the appropriate clone (separated by \*).

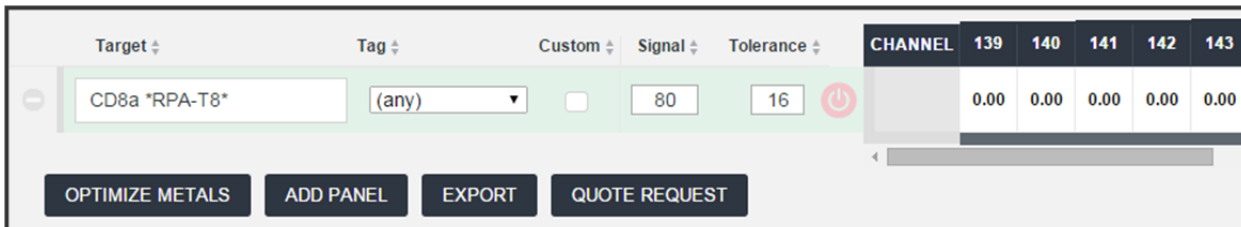
The screenshot shows the Maxpar Panel Designer interface with the target name "CD8a \*RPA-T8\*" entered in the Target field. The Tag dropdown menu is open, showing options: "(any)", "(any)", "146Nd (FDM)", and "162Dy (FDM)". The Signal field is set to 80 and the Tolerance field is set to 16. The "ADD TARGET" button is highlighted. The table on the right shows a row for "162Dy" with values 0, 0, 0, 0, 0.


Choose the tag from the dropdown. This list will be limited to the preconjugates in the catalog. If you choose a metal tag, the preloaded signal and tolerance values will appear for that metal-conjugated antibody.

The screenshot shows the Maxpar Panel Designer interface with the target name "CD8a \*RPA-T8\*" and the tag "162Dy (FDM)" selected. The Signal field is set to 1000 and the Tolerance field is set to 200. The "ADD TARGET" button is highlighted. The table on the right shows a row for "162Dy" with values 0, 0, 0, 0, 0.

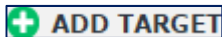
If you choose (any), Optimize Metals will select the best tag from the list of available products for that antibody clone. **Note, Signal and Tolerance will be**

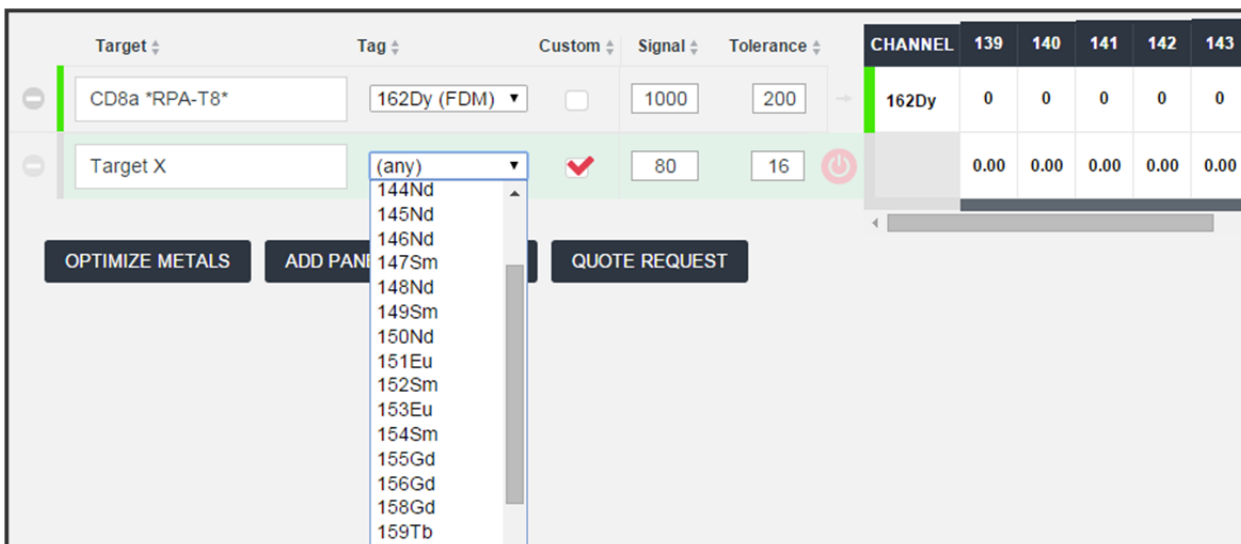
set to their default values (80 and 16 in this example), and these values should be edited after they are empirically determined prior to running **Optimize Metals** (see "Customizing Signal and Tolerance Values" section below).




Click  to activate the antibody in the panel.

## Panel Table: Adding a Custom Antibody

Click  to add a new antibody to the panel. Type the target name in the target box. Check the custom box.



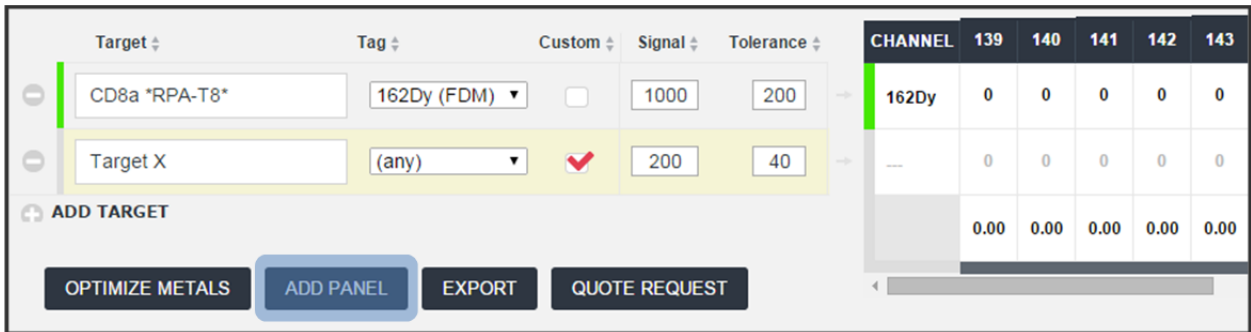
Choose the tag from the dropdown. This list will contain all available Maxpar kit tags. If you choose (any), Optimize Metals will select the best tag from the list. Note, Signal and Tolerance will be set to their default values, and these values should be edited after they are empirically determined prior to running Optimize Metals (see "Customizing Signal and Tolerance Values" section below).

Click  to activate the antibody in the panel.

## Panel Table: Adding a Panel Kit or personal panel

In addition to adding individual antibodies, you can also add Panel Kits and personal panels to the panel you are building. Panel Kits are panels available for purchase from the Fluidigm catalog. Personal panels are panels that you have created and saved within the Panel Designer.

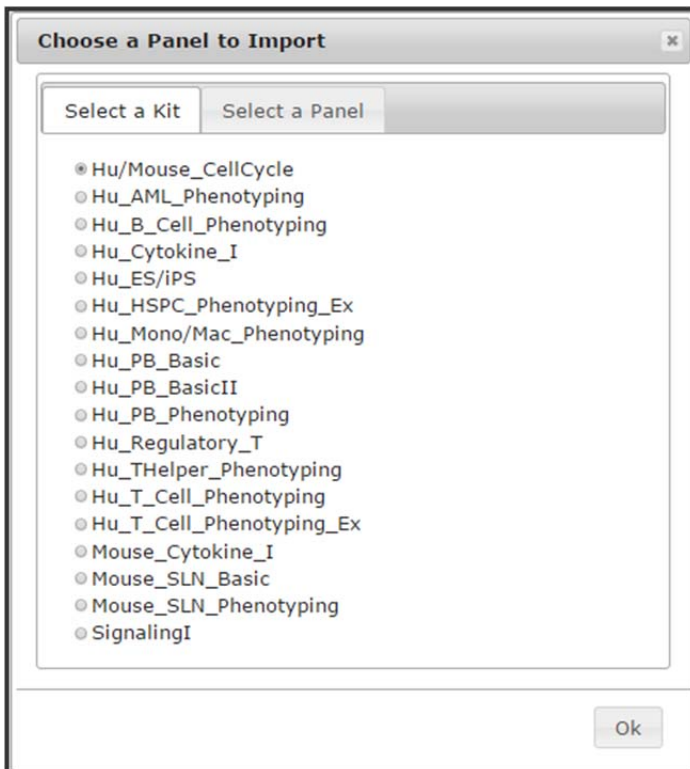
Click **Add Panel** to add a Panel Kit or a personal panel to the existing panel.



The screenshot shows the Panel Designer interface. On the left, there is a list of targets. The first target is 'CD8a \*RPA-T8\*' with a tag of '162Dy (FDM)', a signal of '1000', and a tolerance of '200'. The second target is 'Target X' with a tag of '(any)', a signal of '200', and a tolerance of '40'. Below the targets is an 'ADD TARGET' button. At the bottom, there are four buttons: 'OPTIMIZE METALS', 'ADD PANEL', 'EXPORT', and 'QUOTE REQUEST'. On the right, there is a table with columns for 'CHANNEL' and '139', '140', '141', '142', and '143'. The first row is for '162Dy' and the second row is for '---'. The table shows values of 0 for all channels in the first two rows, and 0.00 for all channels in the third row.

CHANNEL	139	140	141	142	143
162Dy	0	0	0	0	0
---	0	0	0	0	0
	0.00	0.00	0.00	0.00	0.00

The panel import popup will appear. If you want to add a Panel Kit, choose from the list that appears under **'Select a Kit'**, then click **OK**.



The screenshot shows a dialog box titled 'Choose a Panel to Import'. It has two tabs: 'Select a Kit' and 'Select a Panel'. The 'Select a Kit' tab is active, showing a list of panel kits with radio buttons next to each name. The list includes: Hu/Mouse\_CellCycle, Hu\_AML\_Phenotyping, Hu\_B\_Cell\_Phenotyping, Hu\_Cytokine\_I, Hu\_ES/iPS, Hu\_HSPC\_Phenotyping\_Ex, Hu\_Mono/Mac\_Phenotyping, Hu\_PB\_Basic, Hu\_PB\_BasicII, Hu\_PB\_Phenotyping, Hu\_Regulatory\_T, Hu\_THelper\_Phenotyping, Hu\_T\_Cell\_Phenotyping, Hu\_T\_Cell\_Phenotyping\_Ex, Mouse\_Cytokine\_I, Mouse\_SLN\_Basic, Mouse\_SLN\_Phenotyping, and SignalingI. An 'Ok' button is located at the bottom right of the dialog box.

If you want to add a personal panel, choose from the list that appears under **'Select a Panel'**, then click **OK**.



## Panel Table: Interpreting the Signal Overlap Matrix

Once the target is active, the SO matrix table at the right of the screen updates with the SO values corresponding to the newly activated target. Values in the table are expressed in counts, and indicate each tag's (row) estimated signal overlap into each channel (columns). The total received SO for each channel is shown on the bottom row, which is heat-mapped to a percentage of the tolerance value for the target in that channel. Occupied channels have gray background; unused columns have white background. Values in gray text indicate signal overlap between targets expressed exclusively in distinct cell groups (see ["Groups" section below](#)). The heat map bar to the left of the tag reflects SO derived from that tag.

CHANNEL	139	140	141	142	143	144	145	146	147
145Nd	0	0	0	0.21	0.14	2.24		4.13	0
146Nd	0	0	0	0.50	0.30	0.70	2.50		0
142Nd	0	0	0		1.75	0.21	0	0	0
169Tm	0	0	0	0	0	0	0	0	0
163Dy	0	0	0	0	0	0	0	0	0
	0.00	0.00	0.00	0.71	2.19	3.15	2.50	4.13	0.00

## Panel Table: Optimize Metals

The Optimize Metals function determines the best antibody-tag combinations to minimize background in channels containing targets with low tolerance for signal overlap. The algorithm normalizes signal overlap by tolerance, then selects the tags that provide the lowest overall tolerance-adjusted signal overlap. Normalizing by tolerance biases placement of low-tolerance targets in lower background channels and placement of high-tolerance targets in higher background channels.

To use the Optimize Metals function to select tags:

Set the tags for the targets you wish to optimize to (any).

To select from all available tags, check the custom box (see Target X in the example below). To select from the tags available in the Reagent catalog, uncheck the custom box (see CD8a and Ki-67 in the example below).

Target	Tag	Custom	Signal	Tolerance
CD8a *RPA-T8*	(any)	<input type="checkbox"/>	80	16
CyclinB1 *GNS-1*	153Eu (FDM)	<input type="checkbox"/>	2000	400
Ki-67 *B56*	(any)	<input type="checkbox"/>	80	16
pHistoneH3 [Ser28] *HTA28*	175Lu (FDM)	<input type="checkbox"/>	1000	200
pRb [Ser807/811] *J112-906*	150Nd (FDM)	<input type="checkbox"/>	800	160
Target X	(any)	<input checked="" type="checkbox"/>	200	40

ADD TARGET

OPTIMIZE METALS   ADD PANEL   EXPORT   QUOTE REQUEST

In this example, metal conjugated antibodies for CD8a and Ki-67 from the Fluidigm catalog will be selected, and the Signal and Tolerance values corresponding to those products will be used. Custom targets HLA-DR and CD11c are assigned custom tags with default signal and tolerance values.

**NOTE** For custom targets, the signal and tolerance values should be empirically determined and entered prior to running Optimize Metals ([see "Customizing Signal and Tolerance Values" section below](#)). In this example, Target X Signal was set to 200 and Tolerance to 40 as determined by a pilot experiment. For the two non-custom targets set to (any), the Optimize Metals algorithm will use the pre-loaded signal and tolerance values for the metal conjugated antibodies that it evaluates and chooses. For this reason, we left Signal and Tolerance for CD8a and Ki-67 at their default values of 80 and 16.

Click **Optimize Metals**.

Target	Tag	Custom	Signal	Tolerance
CD8a *RPA-T8*	162Dy (FDM)	<input type="checkbox"/>	1000	200
CyclinB1 *GNS-1*	153Eu (FDM)	<input type="checkbox"/>	2000	400
Ki-67 *B56*	172Yb (FDM)	<input type="checkbox"/>	1794	37
pHistoneH3 [Ser28] *HTA28*	175Lu (FDM)	<input type="checkbox"/>	1000	200
pRb [Ser807/811] *J112-906*	150Nd (FDM)	<input type="checkbox"/>	800	160
Target X	165Ho	<input checked="" type="checkbox"/>	200	40

ADD TARGET

OPTIMIZE METALS ADD PANEL EXPORT QUOTE REQUEST

All targets that were originally assigned tags are undisturbed. All tags originally set to (any) have tags assigned to them. Notice that Signal and Tolerance for the custom Target X did not change. In contrast, CD8a and Ki-67 were assigned Signal and Tolerance values corresponding to the Fluidigm antibodies chosen for them.

**NOTE** In some cases, all available catalog tags for a non-custom target conflict with another tag already assigned in the panel. In these cases, Optimize Metals assigns these targets custom tags and provides you with an explanatory notification.

## Panel Wheel

This window is also used to view and edit the selected panel. Each channel tile is arrayed counterclockwise on the wheel in order of ascending tolerance values within four tolerance zones: unassigned (gray), low (4 or less), medium (between 4 and 40), or high (40 or more).

The channel tile height is proportional to the sensitivity of the channel. The tile is heat-mapped to indicate the SO into the channel, expressed as a percentage of the tolerance value for the target in that channel.

The scroller contains tiles for each possible channel. Each tile shows the target, signal and tolerance values and a bar heat-mapped to indicate the SO into the channel, expressed as a percentage of the tolerance value. Targets can be edited or deleted within the scroller. Targets can be added to empty channel boxes or by using the Quick Add box below the scroller.

### 30 Channels

Low

Medium

<input type="text" value="CD69 *FN50"/>	s 80	T 16	175
<input type="text" value="CD11a *HI111"/>	s 80	T 16	174
<input type="text" value="CD38 *HIT2"/>	s 300	T 2	172
<input type="text" value="CD14 *MSE2"/>	s 80	T 16	170
<input type="text" value="CD45 *HI30"/>	s 80	T 16	169
<input type="text" value="CD154 *24-31"/>	s 200	T 40	168

176

139

**Quick Add**

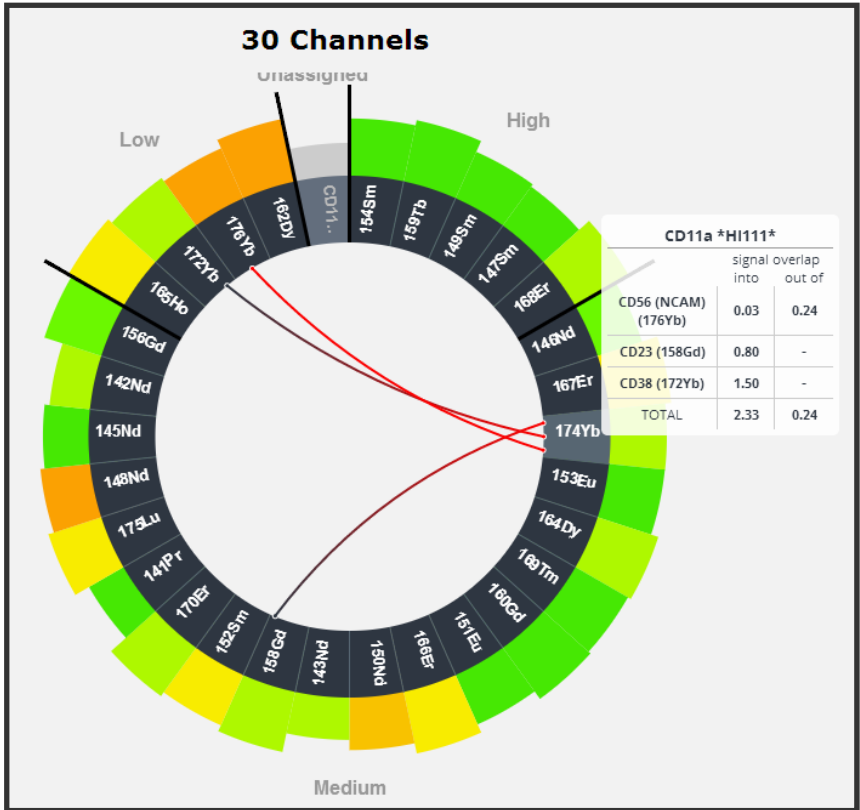
Target:

Tag: (any)  Custom

Signal:  Tolerance:



Click on a tile to reveal lines connecting channels with signal overlap and a table that shows the amount and origin of all SO into the channel and the amount and destination of all SO from the channel.



# Customizing Signal and Tolerance Values

The more accurate the signal values are for each target in the panel, the more accurately the Maxpar Panel Designer will predict signal overlap counts expected in each channel. Furthermore, since tolerance values bias the metal tag choices for targets, it is also important to provide tolerance values appropriate to each target. These values can be edited in the panel table and panel wheel windows.

Each antibody from the Fluidigm reagent catalog has signal and tolerance values predetermined in production, but the probe may perform differently in your experimental system.


**!** **IMPORTANT** For custom targets, the Maxpar Panel Designer assigns a global average for default signal and tolerance values. These values are unlikely to be accurate for your custom antibody. If you have already labeled the antibody, perform a titration to obtain the appropriate values. If you have not labeled the antibody, estimate performance by staining cells with either unlabeled antibody plus metal-conjugated secondary antibody, or FITC antibody plus metal-conjugated anti-FITC.

## Groups

This window is used to manage groups. A group is a subset of targets within a panel expressed on the same cell types. During analysis, cell populations expressing mutually exclusive markers are isolated from one another. As a result, the Optimize Metals algorithm will tolerate an unlimited amount of SO between targets belonging to distinct groups. In this window, you can add or delete groups and assign members to one or more groups.

In the image below, Group A identifies CD8 T cells, B identifies CD4+ T cells, and C identifies B cells. IFN-g and IL17A are common to all three groups. The Optimize Metals function will ignore SO between the following targets:

- CD8 and CD4
- CD4 and CD19
- CD8 and CD19

000 

REACTIVITY: HUMAN

## MANAGE GROUPS

A	B	C
<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>
<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>
<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>
<input type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>

*delete group*

ADD NEW GROUP

### Group A

IL-17A (169Tm)  
IFNg (163Dy)  
CD8a (146Nd )

### Group B

IL-17A (169Tm)  
IFNg (163Dy)  
CD4 (145Nd )

### Group C

IFNg (163Dy)  
CD19 (142Nd)  
IL-17A (169Tm)

## Generating a Quote Request

This window is used to request a quote for the reagents needed to carry out your experiment with the panel. This includes individual metal conjugated antibodies, Panel Kits, Metal Labeling Kits, and custom conjugation services for the targets in your panel. In addition, you can order other reagents necessary for your experiment including sample preparation buffers, barcoding kits, nucleic acid intercalators, IdU, dead cell identifiers, and normalization beads.

To illustrate all of the features of the Quote Request form, consider the following panel that was constructed by adding the Human PB Basic II Panel Kit (green, Fluidigm cat #201315), 143Nd anti-HLA-DR (magenta, Fluidigm cat # 3143013B), and Target X that will be custom conjugated to 165Ho (blue).

	Target ↕	Tag ↕	Custom ↕
⊖	CD4 *RPA-T4*	145Nd (FDM) ▼	<input type="checkbox"/>
⊖	CD16 *3G8*	148Nd (FDM) ▼	<input type="checkbox"/>
⊖	CD8a *RPA-T8*	146Nd (FDM) ▼	<input type="checkbox"/>
⊖	CD14 *M5E2*	160Gd (FDM) ▼	<input type="checkbox"/>
⊖	CD3 *UCHT1*	170Er (FDM) ▼	<input type="checkbox"/>
⊖	CD45 *HI30*	154Sm (FDM) ▼	<input type="checkbox"/>
⊖	CD20 *2H7*	147Sm (FDM) ▼	<input type="checkbox"/>
⊖	HLA-DR *L243*	143Nd (FDM) ▼	<input type="checkbox"/>
⊖	TargetX	165Ho ▼	<input checked="" type="checkbox"/>

Click **Quote Request** on the My Panels, Panel Table, or Panel Wheel window. This will launch the Quote Request form.

**Quote Request**

**Kits**

Qty	Size	Cat#	Name	Target	Clone	Tag	Order Individually
1	25 test	201315	Hu_PB_BasicII_PanelKit				<input type="checkbox"/> y/n
				CD4	RPA-T4	145Nd	
				CD45	HI30	1545m	
				CD3	UCHT1	170Er	
				CD8a	RPA-T8	146Nd	
				CD16	3G8	148Nd	
				CD14	M5E2	160Gd	
				CD20	2H7	1475m	

**Individual Targets**

Qty	Size	Cat#	Name/Target	Clone	Tag	Pre-Conjugate	Labeling Kit	Custom Service Validated	Custom Service Non-Validated
1	100 test	3143013B	HLA-DR	L243	143Nd	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
1	100 test	3999999-4	TargetX		165Ho	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>

**Barcoding (Pd 20-plex)**

Qty:  Size:  Cat #:  Name:  Tag:

**Dead cell identification (103Rh, Cisplatin)**

Qty:  Size:  Cat #:  Name:  Tag:

**Cell identification (Natlr, 127IdU)**

Qty:  Size:  Cat #:  Name:  Tag:

**Sample Prep Buffers (water, Fix I, Perm-S, Cell Staining Buffer, Fix and perm)**

Qty:  Size:  Cat #:  Name:

**Normalization beads (EQ 4 element beads)**

Qty:  Size:  Cat #:  Name:

Panel Kits appear in the top table. Individual antibodies appear in the second table. Accessory reagents appear below the tables.

## Panel Kits

Panel kits are supplied as '25 test' size. Individual antibodies in the kit are listed. By default, quantity is set to '1'. If you do not want to order the kit or any of the antibodies in it, set the quantity to '0'. If you want to order the antibodies individually, check the 'Order Individually' box. This will remove the Kit, and add all of the antibodies in the kit to the Individual Targets table. To undo, click the box corresponding to the kit.

**NOTE** If you changed the tag for a target within the Panel Kit, that target will appear twice in the Quote Request. The undesired tag will appear in the Panel Kit table, while the desired product will appear in the Individual Targets table. In this case, when you click order individually, the target with the undesired tag from the Panel Kit will not be added to the Individual Targets table.

**Quote Request**

**Kits**

ORDER KIT #201315 AS KIT

**Individual Targets**

Qty	Size	Cat#	Name/Target	Clone	Tag	Pre-Conjugate	Labeling Kit	Custom Service Validated	Custom Service Non-Validated
1	100 test ▼	3143013B	HLA-DR	L243	143Nd	●	●	●	●
1	100 test ▼	3999999-4	TargetX		165Ho	●	●	●	●
0	100 test ▼	3154001B	CD45	HI30	154Sm	●	●	●	●
0	100 test ▼	3170001B	CD3	UCHT1	170Er	●	●	●	●
0	100 test ▼	3160001B	CD14	M5E2	160Gd	●	●	●	●
0	100 test ▼	3146003B	CD8a	RPA-T8	146Nd	●	●	●	●
0	100 test ▼	3148004B	CD16	3G8	148Nd	●	●	●	●
0	100 test ▼	3145002B	CD4	RPA-T4	145Nd	●	●	●	●
0	100 test ▼	3147001B	CD20	2H7	147Sm	●	●	●	●

**Barcoding (Pd 20-plex)**

## Individual Targets

Antibodies not part of a Panel Kit are found in this table.

Antibodies from the Fluidigm catalog default to the largest possible test size (100 or 50). You can change to '25 test' using the dropdown menu. If you do not want to order the antibody, set the quantity to '0'. You can also select the 'Labeling Kit' radio button if you want to label the antibody yourself. Metal Labeling Kits are available in '4 Rxn' and '40 Rxn' sizes.

Antibodies not found in the Fluidigm catalog default to 'Custom Service Validated'. You can choose the 'Non-Validated Service' instead. You can also select the 'Labeling Kit' radio button if you want to label the antibody yourself. If you do not want to order the antibody, set the quantity to '0'. Metal Labeling Kits are available in '4 Rxn' and '40 Rxn' sizes.

## Custom Services

Fluidigm offers two kinds of Custom Labeling Services: 'Validated' (Cat #3999999-4, 100 test) and 'Non-Validated' (Cat. #3999999-2, 40 ug). For both services, Fluidigm validates antibody metal labeling. For the 'Validated' service, Fluidigm performs and additional validation in an agreed-upon staining experiment. For both services, you must supply the desired clone in the field provided

## Accessories

For each category, choose the desired experimental accessory from the dropdown in the 'Name' field. Then choose the size and quantity. Click the Plus sign to add an additional product within a category. Click the Minus sign to remove a product

## Submitting the Quote Request

Once the form is completed, click **Quote Request** to generate a PDF.

Customer Information					
John Doe Fluidigm North America john.doe@fluidigm.com 123456789					
Kits					
QTY	SIZE	CATALOG#	NAME	PRICE	
1	25 test	201315	Hu_PB_BasicII_PanelKit	0.00	
Individual Channels					
QTY	SIZE	CATALOG#	PRODUCT	PRICE	
1	100 test	3999999-4	Custom (Val.) - TargetX *ABCD* - 165Ho	0.00	
Barcoding (Pd 20-plex)					
QTY	SIZE	CATALOG#	NAME	TAG	
1	1 kit	201060	20-Plex Pd Barcoding	Pd	
Dead cell identification (103Rh, Cisplatin)					
QTY	SIZE	CATALOG#	NAME	TAG	
1	100 uL	201194	Cisplatin-194Pt	194Pt	
Cell identification (Natlr, 127IdU)					
QTY	SIZE	CATALOG#	NAME	TAG	
1	500 uM	201192B	Intercalator-Ir	natlr	
Sample Prep Buffers (water, Fix I, Perm-S, Cell Staining Buffer, Fix and perm)					
QTY	SIZE	CATALOG#	NAME		
1	50 mL	201065	MaxPar Fix I Buffer		
				<b>Total</b>	<b>0.00</b>

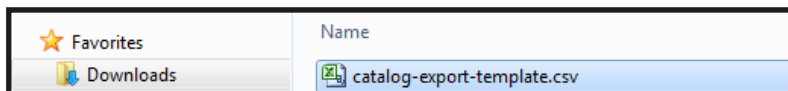
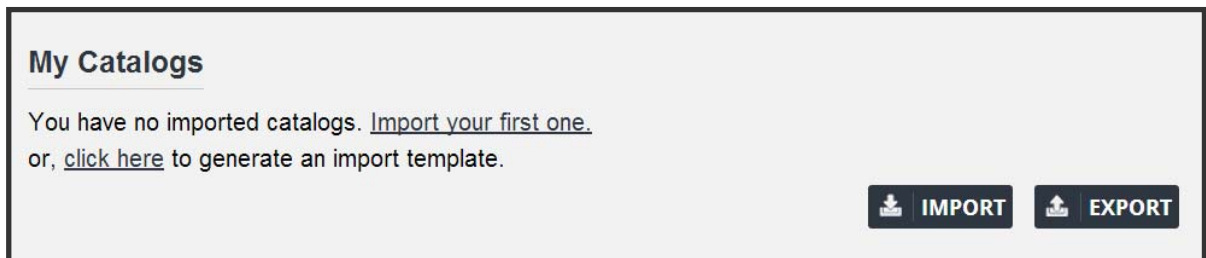
To receive a quote, email this PDF to [sales@fluidigm.com](mailto:sales@fluidigm.com).

# My Catalogs

This window is used to manage your personal catalogs. A catalog is a set of metal-conjugated antibodies that are available for adding to panels. The Maxpar Panel Designer contains all antibodies available on the reagent catalog on the Fluidigm website (FDM) as well as any number of imported personal catalogs. This window shows each personal catalog and the antibodies they contain. You can delete catalogs or share catalogs (csv) using import and export functions.

## My Catalogs: Importing Your First Catalog

Click on the indicated link to generate an import template. This saves a catalog-export-template.csv file into your Downloads folder.



Open the file in excel and complete the fields for each antibody. Be sure to use the tag format indicated below..

Enter the catalog name in column A.

	A	B	C	D	E	F	G	H	I
1	catalog	target	species	clone	tag	signal_di	tolerance	product	cat_num
2	XYZ	CD262	Mouse	MD5-1	159Tb	120	24	159Tb-Ant	XYZ001
3	XYZ	CD73	Mouse	TY/11.8	175Lu	200	10	175Lu-Ant	XYZ002

Save the file in csv format.

Click the **Import** button or the **Import your first one** link.

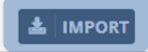
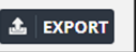
Browse for the catalog and click **Open**.



## My Catalogs: Importing Catalogs

After you have imported your first catalog, you will see it appear in the catalog list.

<input type="checkbox"/> <b>XYZ</b> <a href="#">delete</a>	TARGET	SPECIES	CLONE	TAG	SIGNAL DI	TOLERANCE DI	PRODUCT	CAT. NUMBER	IMPORTED
	CD262	Mouse	MD5-1	159Tb	120	24	159Tb-Anti-Mouse CD262	XYZ001	2014-06-05 14:30:34
	CD73	Mouse	TY/11.8	175Lu	200	10	175Lu-Anti-CD73	XYZ002	2014-06-05 14:30:34

To add additional catalogs, click the **Import** button.

Browse for the catalog and click **Open**.

<input type="checkbox"/> <b>XYZ</b> <a href="#">delete</a>	TARGET	SPECIES	CLONE	TAG	SIGNAL DI	TOLERANCE DI	PRODUCT	CAT. NUMBER	IMPORTED
	CD262	Mouse	MD5-1	159Tb	120	24	159Tb-Anti-Mouse CD262	XYZ001	2014-06-05 14:08:59
	CD73	Mouse	TY/11.8	175Lu	200	10	175Lu-Anti-CD73	XYZ002	2014-06-05 14:08:59

<input type="checkbox"/> <b>ABC</b> <a href="#">delete</a>	TARGET	SPECIES	CLONE	TAG	SIGNAL DI	TOLERANCE DI	PRODUCT	CAT. NUMBER	IMPORTED
	Test	Human	Test	141Pr	100	10	Test	12345A	2014-06-05 14:01:47
	CD4	Human	SK3	174Yb	500	100	CD4	133333	2014-06-05 14:01:47

The new catalog will appear in the catalog list

## My Catalogs: Exporting Catalogs

To export a catalog, check the box next to the catalog name, then click **Export**.  
A csv file will be exported to your Downloads folder.

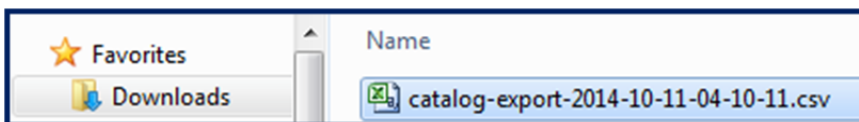
**My Catalogs** IMPORT EXPORT

**ABC** [delete](#)

TARGET	SPECIES	CLONE	TAG	SIGNAL DI	TOLERANCE DI	PRODUCT	CAT. NUMBER	IMPORTED
Test	Human	Test	141Pr	100	10	Test	12345A	2014-06-05 14:42:13
CD27	Human	323	167Er	80	60	CD27	133333	2014-06-05 14:42:13

**XYZ** [delete](#)

TARGET	SPECIES	CLONE	TAG	SIGNAL DI	TOLERANCE DI	PRODUCT	CAT. NUMBER	IMPORTED
CD262	Mouse	MD5-1	159Tb	120	24	159Tb-Anti-Mouse CD262	XYZ001	2014-06-05 14:30:34
CD73	Mouse	TY/11.8	175Lu	200	10	175Lu-Anti-CD73	XYZ002	2014-06-05 14:30:34



## My Catalogs: Deleting Catalogs

To delete a catalog, check the box next to the catalog name, then click **Delete**.  
If you confirm the prompt, the selected catalog will disappear from the list.

## Technical Support

For technical support visit [fluidigm.com/support](http://fluidigm.com/support).



For technical support visit [fluidigm.com/support](https://fluidigm.com/support)