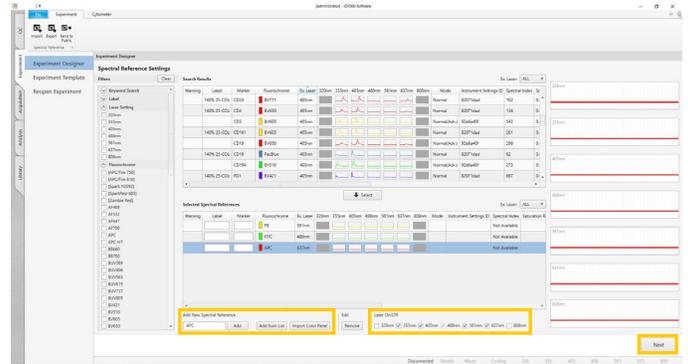


## ID7000™: Adding Spectral References to the Library

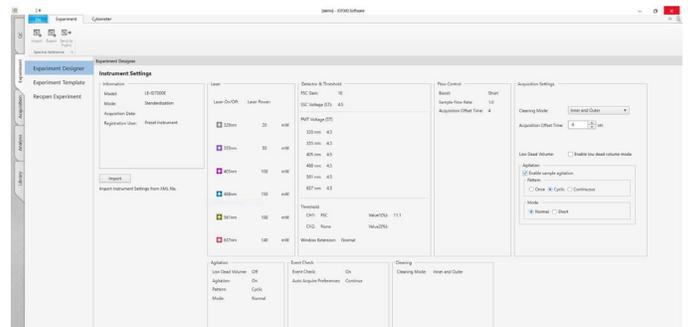
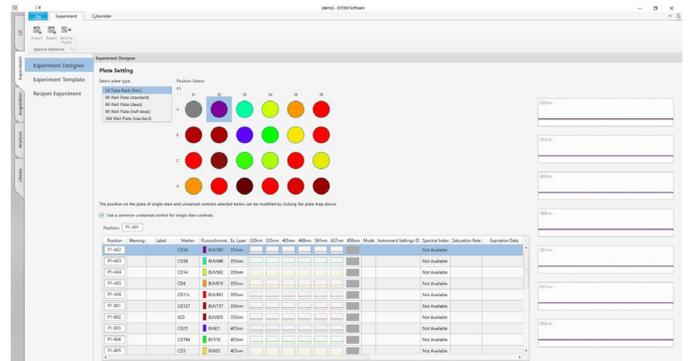
### Add New Single Colors: Three Options

1. In the **Experiment** tab, select the **Experiment Designer** option.
2. *Option 1:* In the bottom half of the screen, in the **Add New Spectral Reference** field, enter each fluorochrome in your experiment and click **Add**.  
*Option 2:* Select **Add from List**. Select the checkbox for each color you would like to add. Click **OK**.  
*Option 3:* Select **Import Color Panel** to import a color list from a CSV file. Click **Browse** to choose your CSV file. The software detects any errors or warnings in your list. Click **Import**.
3. Select the desired lasers. Click **Next**.



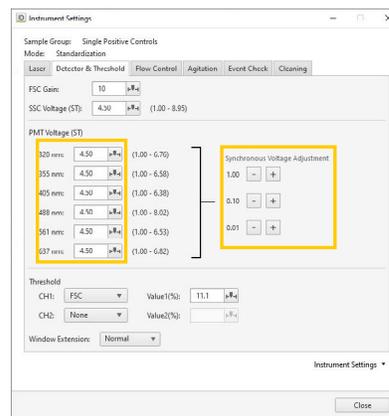
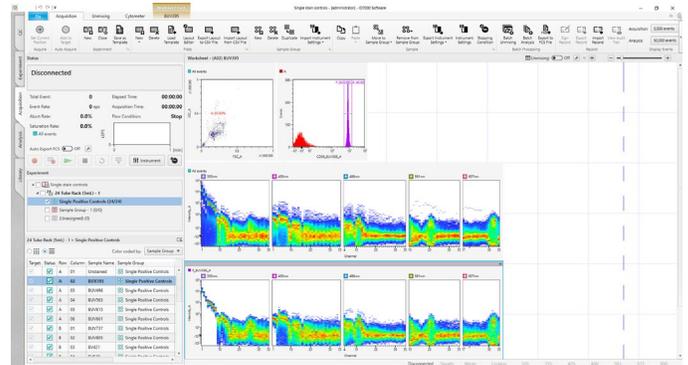
### Use the Experiment Designer

1. Select the desired Tube Rack or Plate type and ensure the samples are in the locations indicated on the layout. Click **Next**.
2. Verify the Instrument Settings. Import settings or adjust Acquisition Settings if desired. Click **Next** in the **Acquisition Settings** window to specify your desired run order. Click **Next**.
3. In the **Information** window, name your experiment and enter any other desired information about the experiment. Click **Next**.
4. In the **Experiment Preview** window, verify all experiment elements. Click **Create Experiment**.



## Record Single Stained Controls

1. Load your single color controls onto the instrument and click **Load** to load the rack or plate.
2. Click **Preview** to view the unstained control, and as needed, adjust FSC, SSC, and Threshold settings. Place the A gate on your population of interest.
3. PMT voltages can be changed simultaneously by using the **Synchronous Voltage Adjustment** or individually using the slider or mouse wheel.
4. If controls are all the same type (beads or cells), right-click the plot and select **Sync Scale and Gate**.
5. Set Stopping Conditions. If using the same stopping conditions for all controls, click **Sync Stopping Condition**.
6. Click **Auto-Acquire** to acquire the Single Color Controls group.
7. Adjust the purple P gates to encompass the positive events for each control in the histogram.



## Calculate Unmixing

1. In the **Unmixing** Tab, click **Unmixing Settings**.
2. Your unstained control is applied under the Negative column for each control, and each P gate is assigned to its respective control under the Positive column. Click **Calculate**.
3. If any single color controls were recorded with saturated data, select the checkbox that allows the software to calculate the unmixing without these saturated events. If a caution mark appears in the Spectral Reference (SR) column, hover over it to display the reason the SR was flagged.
4. Once the software has calculated the spectral reference, it asks if you want to apply the spectral reference to other sample groups with the same laser settings. Click **Apply**.



| Index | Marker | Fluorochrome | Ex. Laser | Mode | SR | Negative        | Positive             |
|-------|--------|--------------|-----------|------|----|-----------------|----------------------|
| 1     | CD56   | BUV395       | 355nm     | Std. | ●  | ● A (Unstained) | ● P_BUV395_A (BUV... |
| 2     | CD38   | BUV496       | 355nm     | Std. | ●  | ● A (Unstained) | ● P_BUV496_A (BUV... |
| 3     | CD10   | BUV563       | 355nm     | Std. | ●  | ● A (Unstained) | ● P_BUV563_A (BUV... |
| 4     | CD19   | BUV615       | 355nm     | Std. | ●  | ● A (Unstained) | ● P_BUV615_A (BUV... |

| Index | Marker | Fluorochrome | Ex. Laser | Mode | SR | Negative        | Positive             |
|-------|--------|--------------|-----------|------|----|-----------------|----------------------|
| 1     | CD56   | BUV395       | 355nm     | Std. | ●  | ● A (Unstained) | ● P_BUV395_A (BUV... |
| 2     | CD38   | BUV496       | 355nm     | Std. | ●  | ● A (Unstained) | ● P_BUV496_A (BUV... |
| 3     | CD10   | BUV563       | 355nm     | Std. | ●  | ● A (Unstained) | ● P_BUV563_A (BUV... |
| 4     | CD19   | BUV615       | 355nm     | Std. | ⚠  | ● A (Unstained) | ● P_BUV615_A (BUV... |
| 5     | CD3    | BUV661       | 355nm     | Std. | ●  | ● A (Unstained) | ● P_BUV661_A (BUV... |
| 6     | CD127  | BUV737       | 355nm     | Std. | ●  | ● A (Unstained) | ● P_BUV737_A (BUV... |

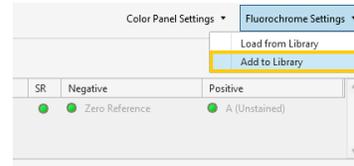


## Store Spectral References in the Library

- Once your Spectral References are calculated and look good, select **Fluorochrome Settings** from the menu in the **Unmixing Settings** window. Select **Add to Library**. (You can also click **Add to Library** right from the **Unmixing** tab in the main software screen.)
- In the **Add to Library** window, enter desired Marker names as well as a group Label for filtering and easy navigation in the library.

**Tip:** To add a label for all samples at the same time, highlight all the fluorochromes and type in the desired label name in any one dialog box.

- Click **Add to Library**.



**Add to Library**

Check to see if there are any warning sign(s).  
 If warning sign(s) are present, deselect that single positive control(s).  
 On selected control(s) fill in reagent information.

| Select                              | Warning | Label | Marker | Fluorochrome | Ex. Laser | 320nm | 355nm | 405nm | 488nm | 561nm | 637nm | 808nm | Mode | Instrument Settings ID | Spectral Index | Saturat |
|-------------------------------------|---------|-------|--------|--------------|-----------|-------|-------|-------|-------|-------|-------|-------|------|------------------------|----------------|---------|
| <input checked="" type="checkbox"/> |         |       | CD56   | BUV395       | 355nm     |       |       |       |       |       |       |       | Std. | 8b94dc-dd              | 399            | 0.0%    |
| <input checked="" type="checkbox"/> |         |       | CD38   | BUV496       | 355nm     |       |       |       |       |       |       |       | Std. | 8b94dc-dd              | 121            | 0.0%    |
| <input checked="" type="checkbox"/> |         |       | CD10   | BUV563       | 355nm     |       |       |       |       |       |       |       | Std. | 8b94dc-dd              | 89             | 0.0%    |
| <input checked="" type="checkbox"/> |         |       | CD19   | BUV615       | 355nm     |       |       |       |       |       |       |       | Std. | 8b94dc-dd              | 136            | 0.0%    |