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Quick Reference Guide

Plotting IC_{50} with the RTCA Software

This quick reference guide describes the procedure for calculating IC_{50}s from Cell Index data, using RTCA Software PRO v2.0 and newer (5454433001).

Before calculating the IC_{50}, it is recommended that the Cell Index be normalized. In Figure 1, the treatments were added at approximately 19 hours, where all the curves, except the blue one, begin to decrease. There are some differences in the Cell Index even before this time, as shown by the blue arrows, due to pipetting differences and edge effect. Normalizing to the time point just before the treatments are added corrects this (Figure 2).

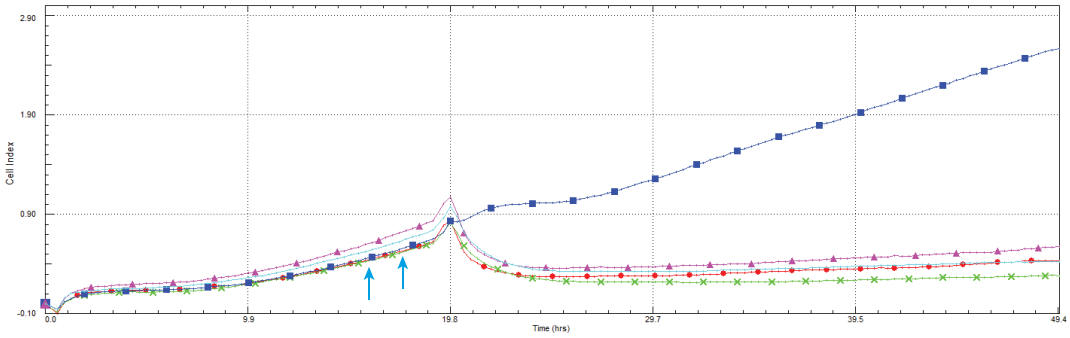

Figure 1. Pre-effector differences in Cell Index.

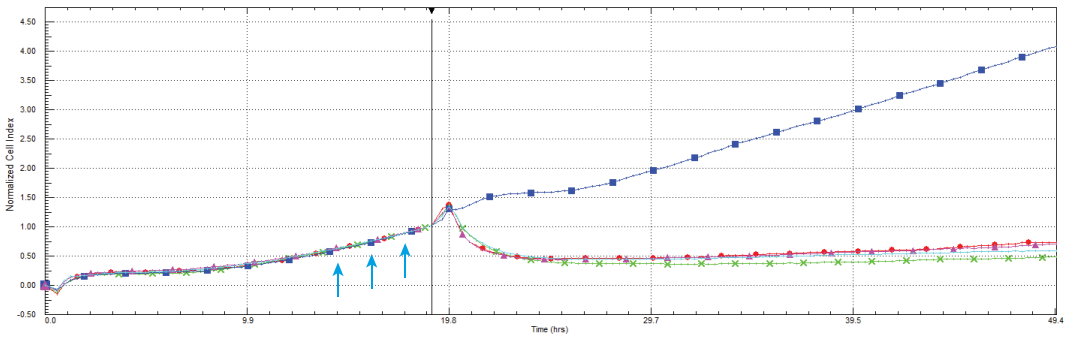

Figure 2. Normalization corrects discrepancies due to experimental error.

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1. Choose the wells of interest and plot them.
2. Before calculating the cytolysis, the Cell Index must be normalized. To find the correct point to start normalization, look for the point where the curve usually has a small bump or change in direction. When the plate is removed and effectors are added, the plate temperature changes, and the cells slightly shrink or expand, depending on the cell type. This causes a change in the Cell Index. Drag a square around that region with your mouse to zoom in (Figure 3).
3. Once you have zoomed in, you can change to Normalized Cell Index (Figure 4A). Drag the black arrowhead (Figure 4B) or use the drop-down menu (Figure 4C) to choose the point just before the bump or direction change in the trace.

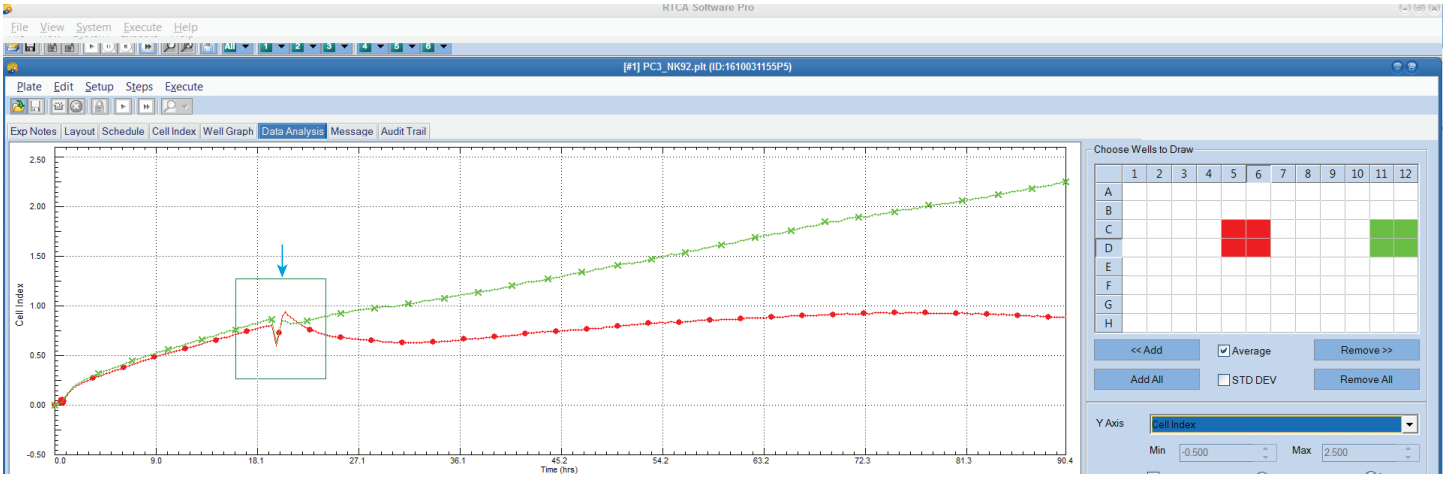

Figure 3. Selecting an area to zoom.

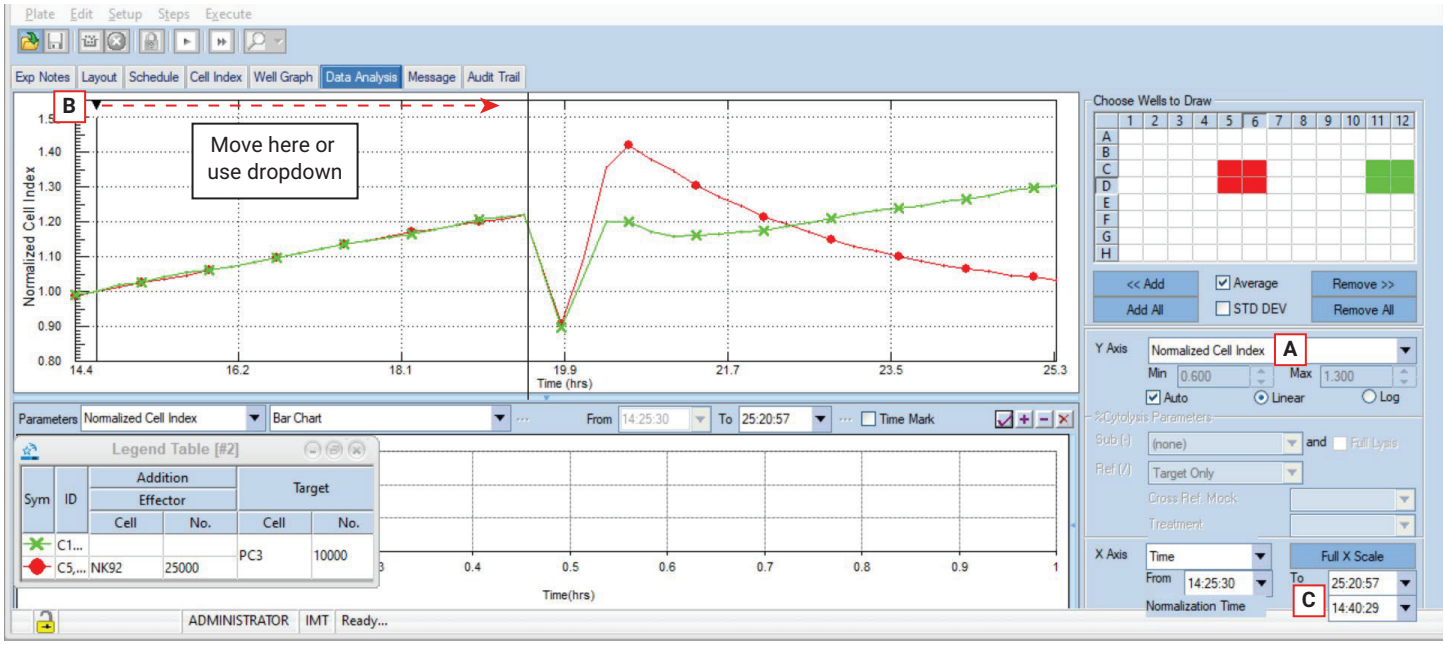

Figure 4. (A) Select "Normalized Cell Index" from the Y Axis drop-down menu. (B) Drag the black arrowhead, or (C) select a time point at which to start normalization.

4. **Using the drop-down:** The software will detect breaks in the readings and mark them in a different color (Figure 5). The value in the pink font corresponds to the first reading after the plate was returned to the device (i.e. after adding the treatments). Normalize at the time point just before the highlighted time, which corresponds to the last time when all the conditions are theoretically identical (that is, just before the treatments are added).

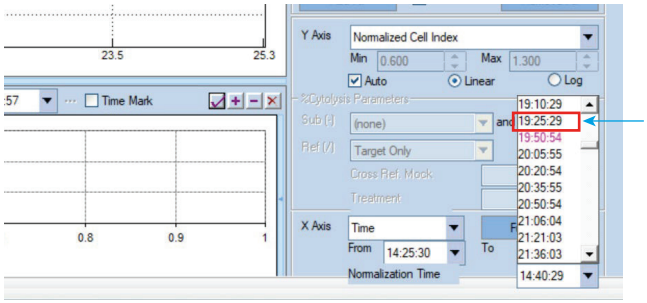

Figure 5. Select the time point just above the highlighted one.

5. Click **Full X Scale** to again visualize the whole plot (Figure 6).

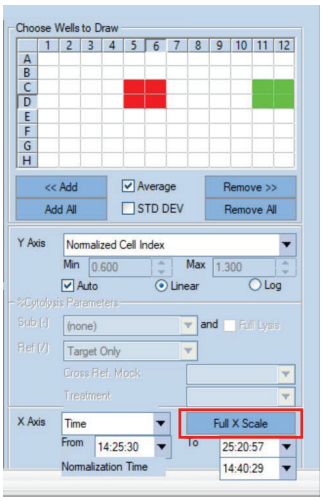

Figure 6. The **Full X Scale** button.

The normalization process is now complete.

6. Select **DRC** (Dose Response Curve) (Figure 7A) in this drop-down. By default, the analysis is run using the last data point collected. To choose a different time, use the "To" drop-down menu (Figure 7B).

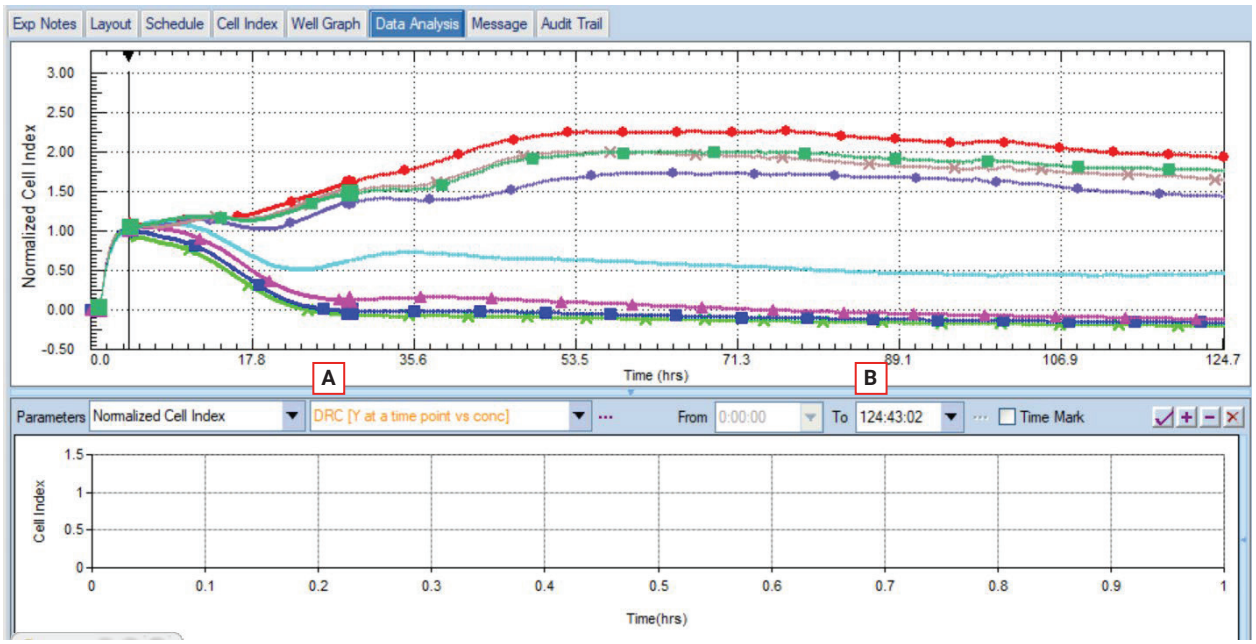

Figure 7. (A) Select **DRC** from the drop-down menu. (B) The time range drop-down menu.

– Alternately, check the Time Mark box (Figure 8A). This will result in the display of a red arrowhead and line on the right edge of the top graph (Figure 8B). Drag the arrowhead to the time point of interest.

7. Go to the chart region of the page. To select the type of fit, click the **...** icon (Figure 9A), then use the Formula drop-down menu that pops up (Figure 9B).

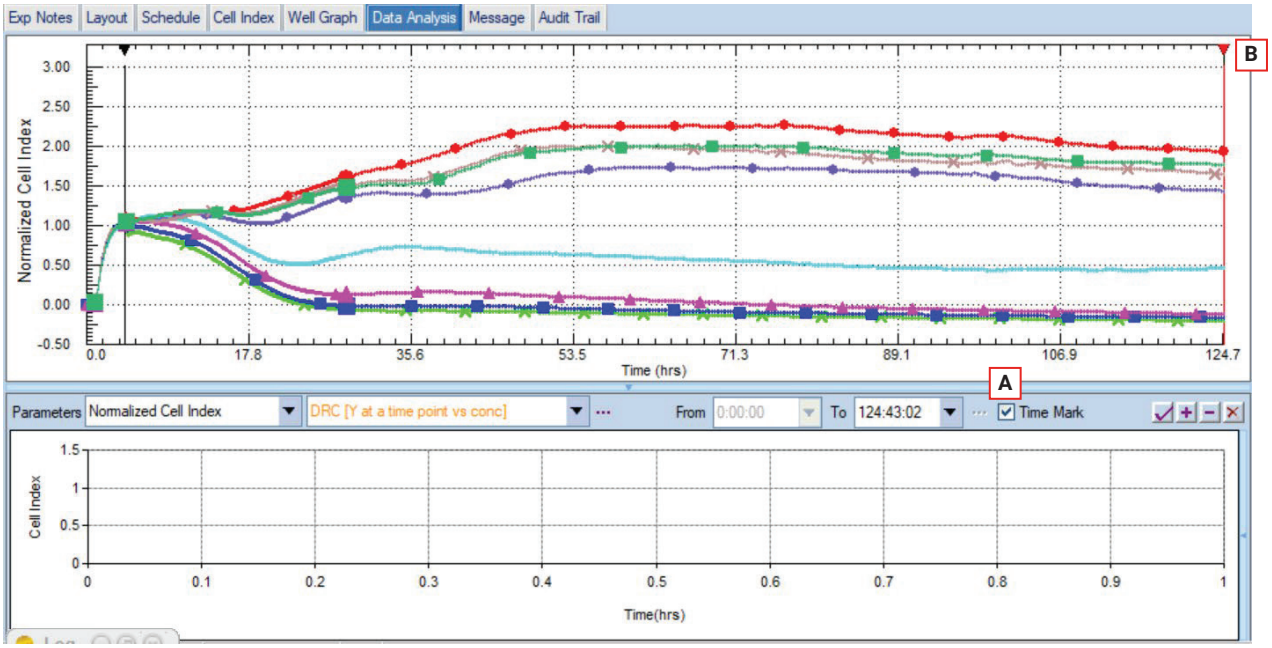

Figure 8. (A) The Time Mark checkbox. (B) The time point slider.

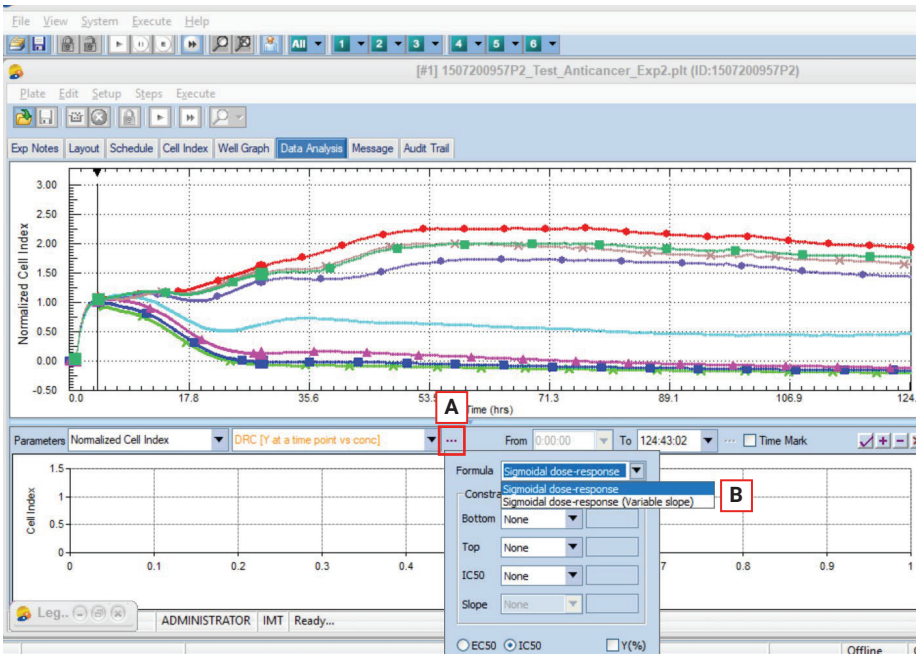

Figure 9. (A) The menu icon for curve fitting. (B) The Formula drop-down menu.

8. Click on the check mark to generate the plot (Figure 10A). To compare other time points or compounds, use the "+" button to add additional plots to the same graph (Figure 10B).

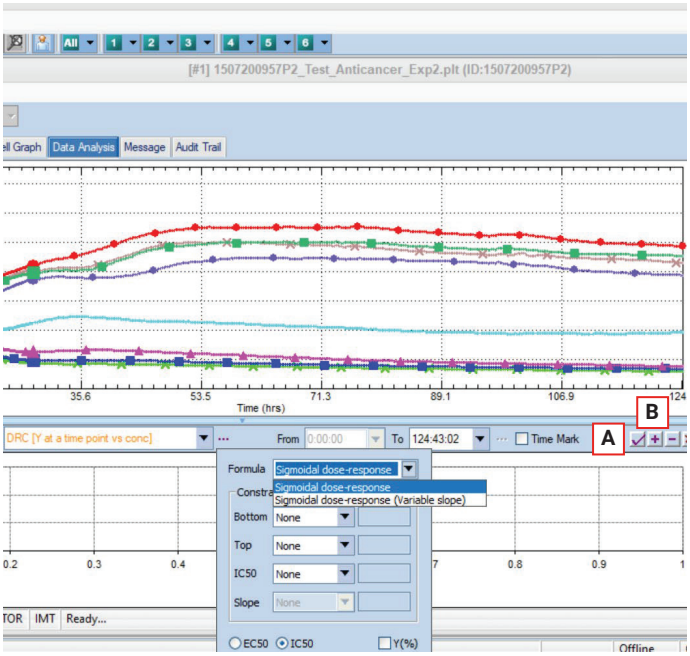

Figure 10. (A) **Check** button. (B) **Add** button for adding data to the current chart.

9. To display the IC_{50} value and R^2 table, click the blue arrowhead indicated in Figure 11.

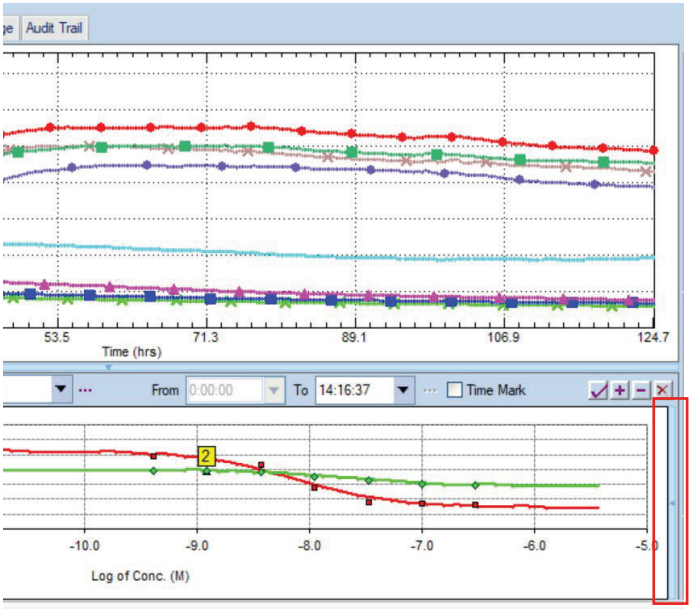

Figure 11. The arrow button that displays the IC_{50} values and R^2 table (indicated with a red rectangle).

The IC_{50} table is displayed (Figure 12).

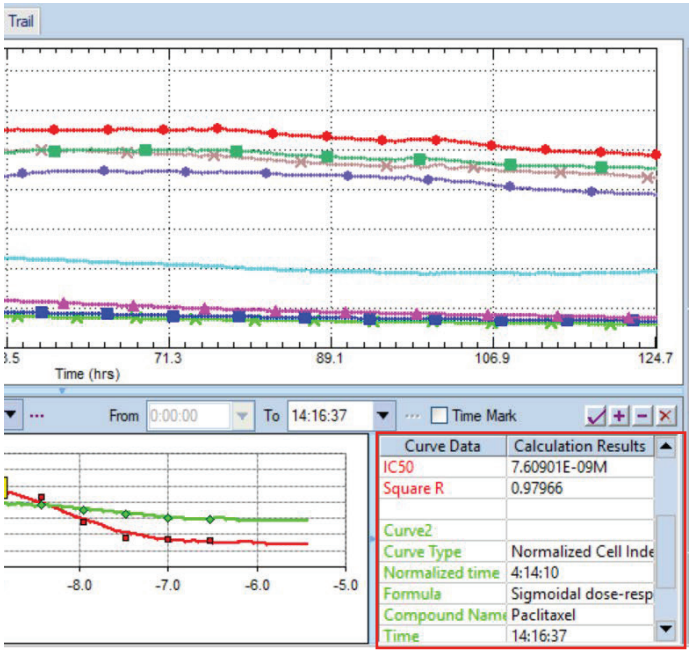

Figure 12. IC_{50} table.

To export the data:

- Right-click on either graph and select **Copy Data**, *OR*
- Select **Plate > Export Experiment Info** (Figure 13).

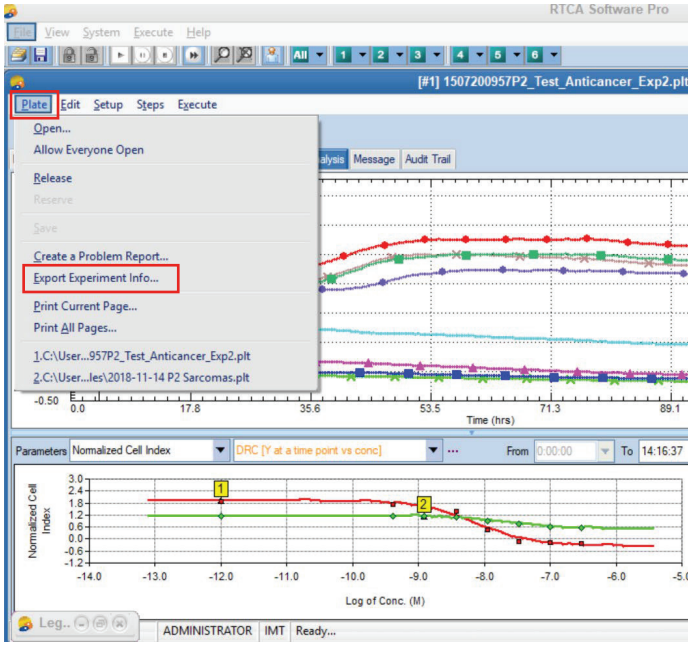

Figure 13. The Export command in the Plate menu.

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