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## **MSD QuickPlex SQ 120 Quick-Start Guide**

### **Acquisition and Analysis Software: MSD Discovery Workbench**

**1 plate = 1.5 minutes to read**

#### **Instrument Daily QC with the Electronic Test Plate:**

Each QuickPlex SQ 120 system is shipped with an Electronic Test Plate. This test plate verifies operation of the system and can be used for operational qualification. It does not verify performance and should not be used for calibration purposes or performance qualification.

1. Open MSD Discovery Workbench software.
2. Click the Status button or go to Tools→Status and check that the CCD camera is cooled to -25°C +/- 2°C and says "Ok!" If it is out of range, try re-starting the software or re-booting the computer.
3. Select Read from Bar Code on the Plate Type Menu.
4. Leave the Apply Kit Layout box unchecked.
5. Check Read and enter 1 in the text field.
6. Load the electronic test plate into the input stack. Note that if an assay plate is installed backwards, sensors in the instrument will automatically identify the plate orientation and adjust the data acquisition and analysis accordingly.
7. Click the Run button in the Instrument window.
8. Run Name is optional.
9. Click OK to start the plate read.
10. View the status in the lower left region (Status bar) of the software to monitor progress. When the plate read is complete, the electronic plate is ejected to the output stack and Run is enabled again.
11. Wells A9, B10, C11, D12, E4, F3, G2, and H1 should display numbers above 2000. All other wells should have just a background signal. This is easiest to see by selecting View→Layer Views→Color Map.

#### **Reading an Assay Plate**

1. From the Plate Type menu, select Read from Bar Code or your plate configuration from the dropdown menu.
2. Leave the Apply Kit Layout box unchecked.
3. Leave Return Plates to Input Stack unchecked.
4. Select the Read checkbox and enter the number of plates to be read.
5. Optional: Select Partial Plate if only part of the plate is to be read (only with plate configuration Plate Type).
6. Click Run.
7. Optional: Run Name is useful when a number of plates will all be read together that are part of a single experiment, enabling quick lookup of data from groups of related assay plates. This name will be labeled on the Plates (Table) tab in the Plate Data History and may be assigned as the custom exported file name. Example labels: study name, project ID, etc.
8. Plate your assay plate in the input stack.
9. Click OK to start the plate read.



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### Plate Layout – Standards and Samples in Duplicate

1. Click on Plate Data History button or go to Tools→Plate Data History.
2. Click New Plate Layout in Library button.
  - a. Choose appropriate Spot Layout (1, 4, 10-spot).
  - b. Name the layout with the MSD kit name.
  - c. Click New.
3. Click the Assign Assays button “A”.
  - a. Refer to your kit protocol for the list of spot assignments.
  - b. Right-click a spot to create or assign assay or choose a pre-defined assay From Kit Layout.
4. Highlight your standard columns and click the red Assign Standards button.
  - a. Change number of replicates to 2, and choose Replicate Right.
  - b. For V-plex MSD kits, uncheck Apply to All Assays.
  - c. From the kit Certificate of Analysis (COA), enter the top Starting Concentration of the selected analyte and the Concentration Dilution Factor.
  - d. Click Dilute Down.
  - e. Change the lowest standard to zero.
5. Highlight the first two columns of your unknowns and click the yellow Assign Unknowns button.
  - a. Change number of replicates to 2, and choose Replicate Right.
  - b. Enter your sample dilution factor under Starting Dilution and enter 1 for Sample Dilution Factor.
  - c. Click Dilute Down.
6. Repeat step 6 for all remaining duplicate columns.
7. Set up 96-well grid in Excel and enter the sample names, including replicates. Replicates must have identical sample names. Non-replicates must have unique sample name. See image below:

	1	2	3	4	5	6	7	8	9	10	11	12
<b>A</b>	Calib 1	Calib 1	49384	49384	51970	51970	50631	50631	49691	49691	49567/8 G	49567/8 G
<b>B</b>	Calib 2	Calib 2	49599	49599	52739	52739	51093	51093	49896	49896	49567/8 F	49567/8 F
<b>C</b>	Calib 3	Calib 3	49786	49786	52957	52957	49438	49438	50116	50116	49771	49771
<b>D</b>	Calib 4	Calib 4	50003	50003	49400	49400	49656	49656	50355	50355	50004	50004
<b>E</b>	Calib 5	Calib 5	50252	50252	49606	49606	49858	49858	50580	50580	50233	50233
<b>F</b>	Calib 6	Calib 6	50530	50530	49798	49798	50065	50065	50791	50791	50466	50466
<b>G</b>	Calib 7	Calib 7	50749	50749	50169	50169	51083	51083	51006	51006	50954	50954
<b>H</b>	Calib 8	Calib 8	50963	50963	50417	50417	49453	49453	51321	51321	49736	49736

- a. Select the region containing just the sample names, and copy (CTRL-C).
  - b. In the Plate Layout Editor, highlight the corresponding cells, right-click and select Paste Sample Names from Clipboard.
  - c. Click Assign Unit button and check that the correct unit is being used (usually pg/ml).
  - d. Close Plate Layout Editor and save layout.
8. Right-click the plate to analyze and select Analyze Plates.
  9. Select the appropriate Plate Layout from the drop-down list. This will create an Experiment (raw plate data + layout).



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## Experiment Analysis in Excel

1. In the Plate Data History, choose Experiments tab.
2. Open your Experiment. Click on the title, right-click and choose Rename. Change the default name of your Experiment (Experiment\_date\_time) to something meaningful, such as your plate run name (if you are only running a single plate) or overall experiment/project/protocol name (if you will be running several plates over time you want to group together). You may have to close the Plate Data History for the name to update in the list.
3. The second option under your plate name is the Plate Layout. Check that your standards, dilution factors, and samples names are all correct.
4. To add another plate into this Experiment, open the Plates (Table) view side-by-side with your Experiment. Then drag the desired plate into your existing experiment. It is useful to give each plate a meaningful name to organize your data.
5. *Optional:* Right click on the open Experiment title, select Create Plot. Rename new plot "All Standards".
  - a. Drag the Standards and Unknowns from each analyte to the new plot.
  - b. Right-click in plot, and select Show Detection Range for each standard curve.
6. Click in main body of Experiment, and save.
7. *Optional:* Open a new workbook in Excel, copy your All Standards plot to the first sheet and name it "Standards". Underneath the plot, make a table of your LLOD and ULOD for each analyte from the Calc. Low and High values indicated on the plot.
8. Open your Experiment's Plate Data Table. Drag the Assay column to the first column.
9. Right-click at the top of the columns, Copy Table to Clipboard and then paste the data into a new sheet in Excel workbook. Name this sheet "Exported Data".
10. Keeping the data selected, click Insert→Pivot Table. Click OK.
11. Drag the following fields to the following areas:
  - a. Assay → Columns
  - b. Sample → Rows
  - c. Calc. Conc. Mean → Values
12. Right-click on the Pivot Table title "Count of Calc. Conc. Mean", select Value Field Settings. Select Average. Click OK.
13. Click the Row Labels drop-down, and uncheck all of the standards. Click OK.
14. Right-click on the Pivot Table title "Average of Calc. Conc. Mean", select Pivot Table Options. Select the Totals & Filters tab and uncheck the two Grand Totals boxes. Click OK. Re-name this new sheet "Pivot Table".
15. *Optional:* Create a new sheet and label it "LOD" for your limits of detection.
  - a. Copy and paste the contents of the Pivot Table to this sheet as values only.
  - b. Copy and paste the LLOD/ULOD values from the "Standards" sheet underneath the sample data, making sure all analytes are in the same order as the data columns. Multiply the LLOD/ULOD values by your sample dilution factor.
  - c. Center all columns.
  - d. Format your sample concentrations to 2 decimal places.
  - e. Conditional format each data column:
    - i. Select the range of data from one analyte column.
    - ii. Select Home→Conditional Formatting→New Rule.
    - iii. Select "Format only cells that contain". Choose Cell Value, **less than**, and choose your LLOD value for that analyte. Format the font to **blue**. Change "LLOD" in the sheet to the same **blue** color.
    - iv. Select the range of data from the same analyte column.
    - v. Select Home→Conditional Formatting→Manage Rules.
    - vi. Add a New Rule.
    - vii. Select "Format only cells that contain". Choose Cell Value, **greater than**, and choose your ULOD value for that analyte. Format the font to **red**. Change "ULOD" in the sheet to the same **red** color.