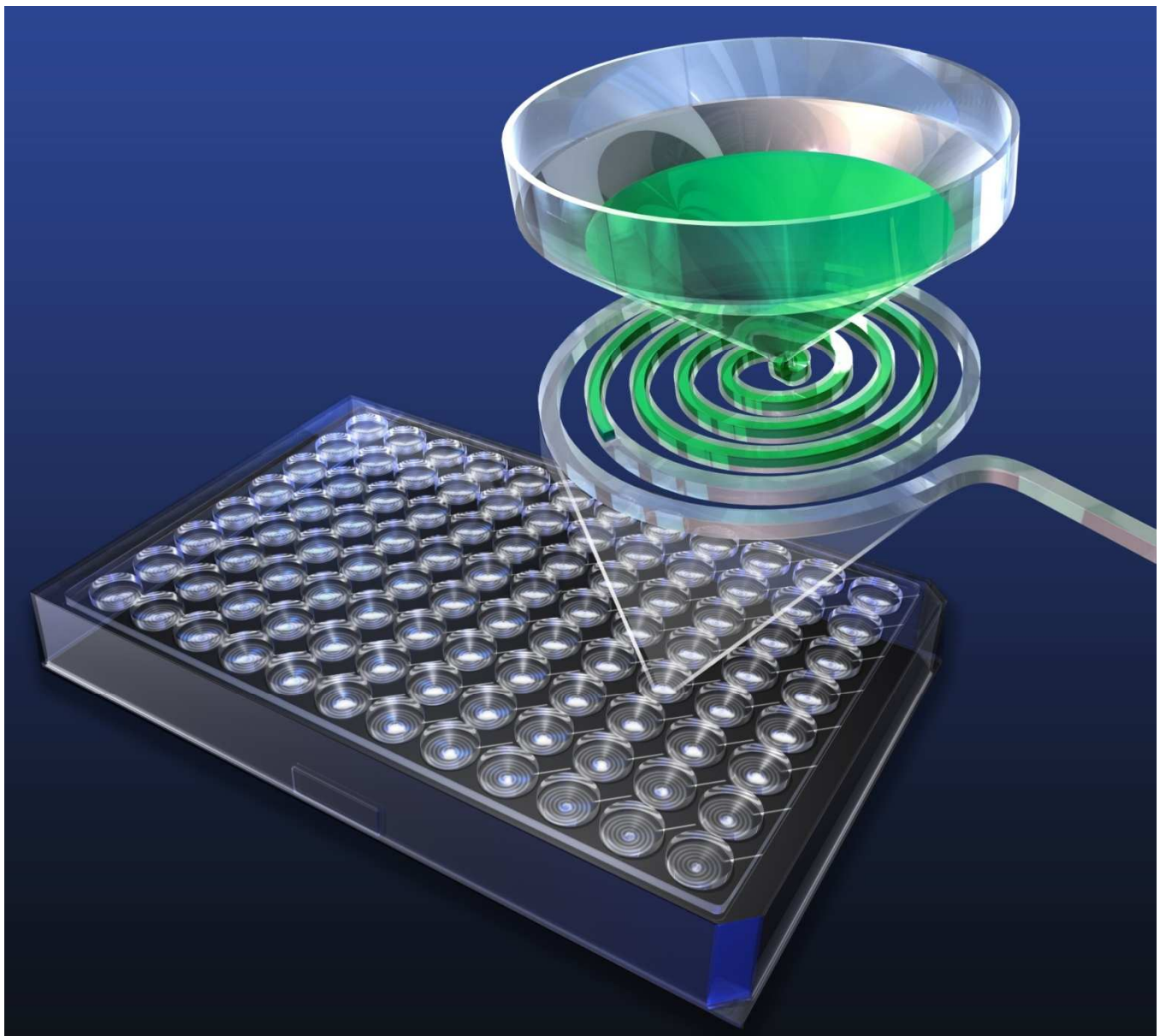


TECHNICAL NOTE

**TN0003: Optimiser™ Microplate System (ELISA) Setup Guide
on the Tecan Infinite® M200 Microplate Reader**



*Better Immunoassays through
Innovative Microfluidics*

READER SETUP WITH TECAN INFINITE® M200 :

The TECAN Infinite® M200 Multi-mode Microplate Reader with fluorescence function has been tested for compatibility with Siloam's Optimiser™ microplate System in ELISA assay with OptiGlow™ chemifluorescent substrate. Please refer to the Optimiser™ Technology page on Siloam's website for more details on the principles behind the Optimiser™ microplate platform. For more detailed information of Infinite® readers or software, please contact Tecan technical support.

Recommended Optics

	Wavelength
Excitation	544* nm
Emission	590 nm

**There is no significant difference using excitation wavelength at 529nm or 544nm in this reader.*

Instrument Setup

Turn on the plate reader, and open up Tecan i-control software on computer.

Step 1- Select the Measurement Method

In panel of "Measurements" on the left side of screen, select "**Fluorescence Intensity**". And input "544" for Excitation and "590" for Emission.

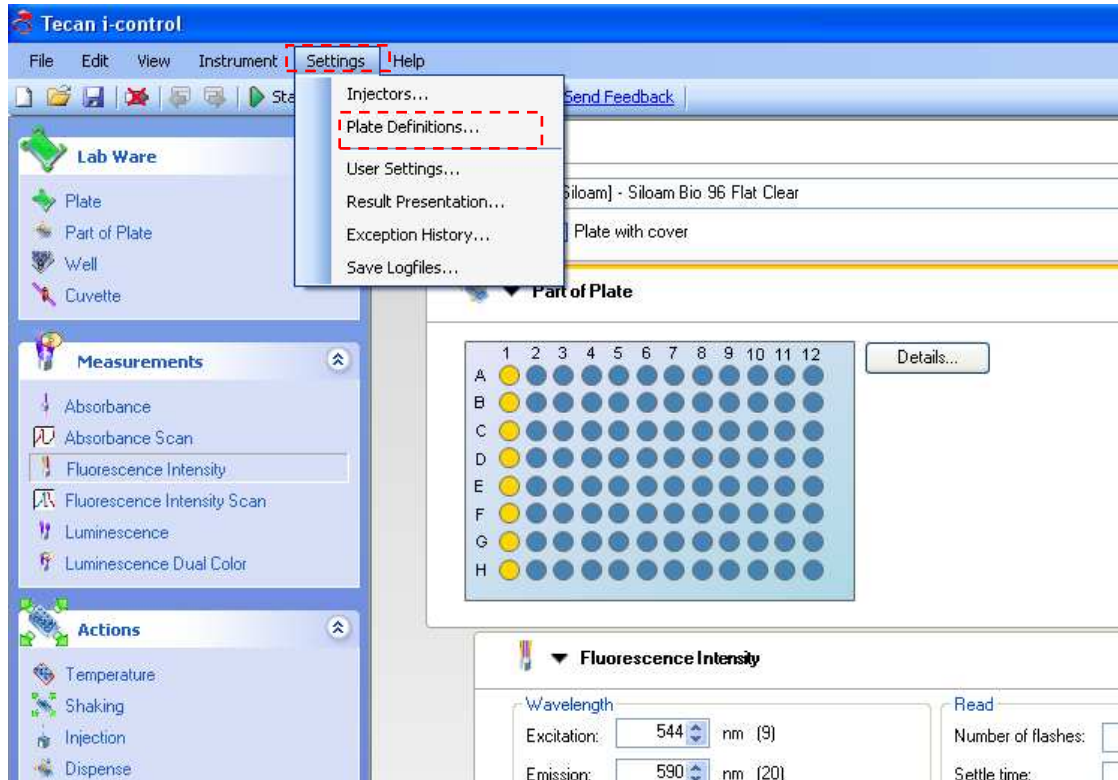
The screenshot displays the Tecan i-control software interface. On the left sidebar, the "Measurements" panel is expanded, and "Fluorescence Intensity" is selected, highlighted with a red dashed box. Below this, the "Fluorescence Intensity" configuration panel is visible, also highlighted with a red dashed box. It shows the following settings:

- Wavelength: Excitation: 544 nm (9), Emission: 590 nm (20)
- Read: Number of flashes: 25, Settle time: 0 ms
- Gain: Manual: 67
- Mode: Top (selected), Bottom

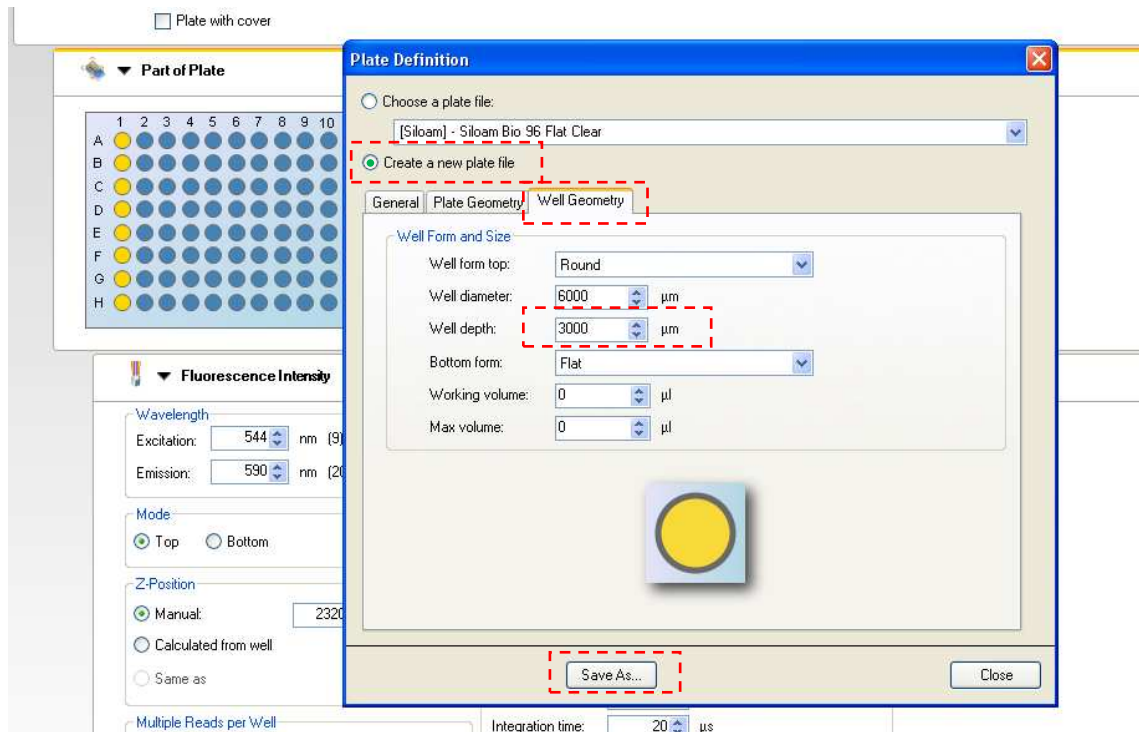
The main window shows a 96-well plate layout (A-H, 1-12) and a "Part of Plate" selection area.

Step 2- Select the Plate Type and Optic Probe Direction

Select “**Top**” for Modes. Select the “**setting**” and click “**Plate Definitions**”



In panel of Plate definition, select “**Create a new plate file**”, then select “**Well Geometry**”, input “**3000**” in the “**Well depth**”, leave everything else as default setting, then **save** as a plate definition.



Step 3- Select the Z-Position

Tecan Infinite® M200 provides the Z-position adjustment to achieve best optical reading. To select the Z-position, an Optimiser™ Plate will be used with one well containing positive control solution

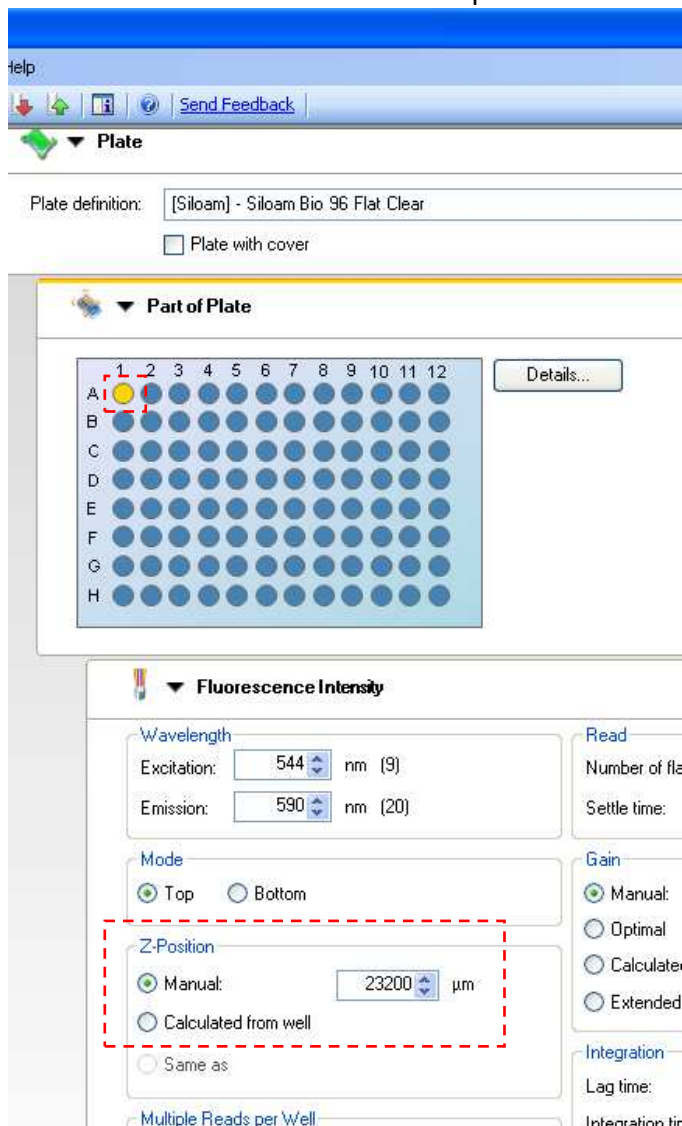
Material:

- 1) Siloam's SAV-HRP (cat# OMR-HRP). *Note: Any HRP conjugate with concentration greater than 1 µg/mL can be used for this test with following experimental protocol*
- 2) Siloam's OptiGlow™ substrate.
- 3) One Optimiser™ Microplate.

Positive Control Preparation and Loading:

In a clean plastic tube, add 50 µL of OptiGlow™-A, 50 µL of OptiGlow™-B, 1 µL of OptiGlow™-C, and 1 µL of supplied SAV-HRP stock solution, mix well, and wait for 2 minutes. The substrate will be fully developed and stable for hours. Load 4 µL of mixture into one well (*well A1 is used in this instruction*) of Optimiser™ microplate and wait until the well is empty (do not use pad/holder)

Use function of “**Calculated from well**” with well A1. The reader will read the signal in various Z-position and give the optimal Z-position. Save or record the number for all Optimiser™ measurement. Please refer to Tecan's Reader User Instruction for detail operation.

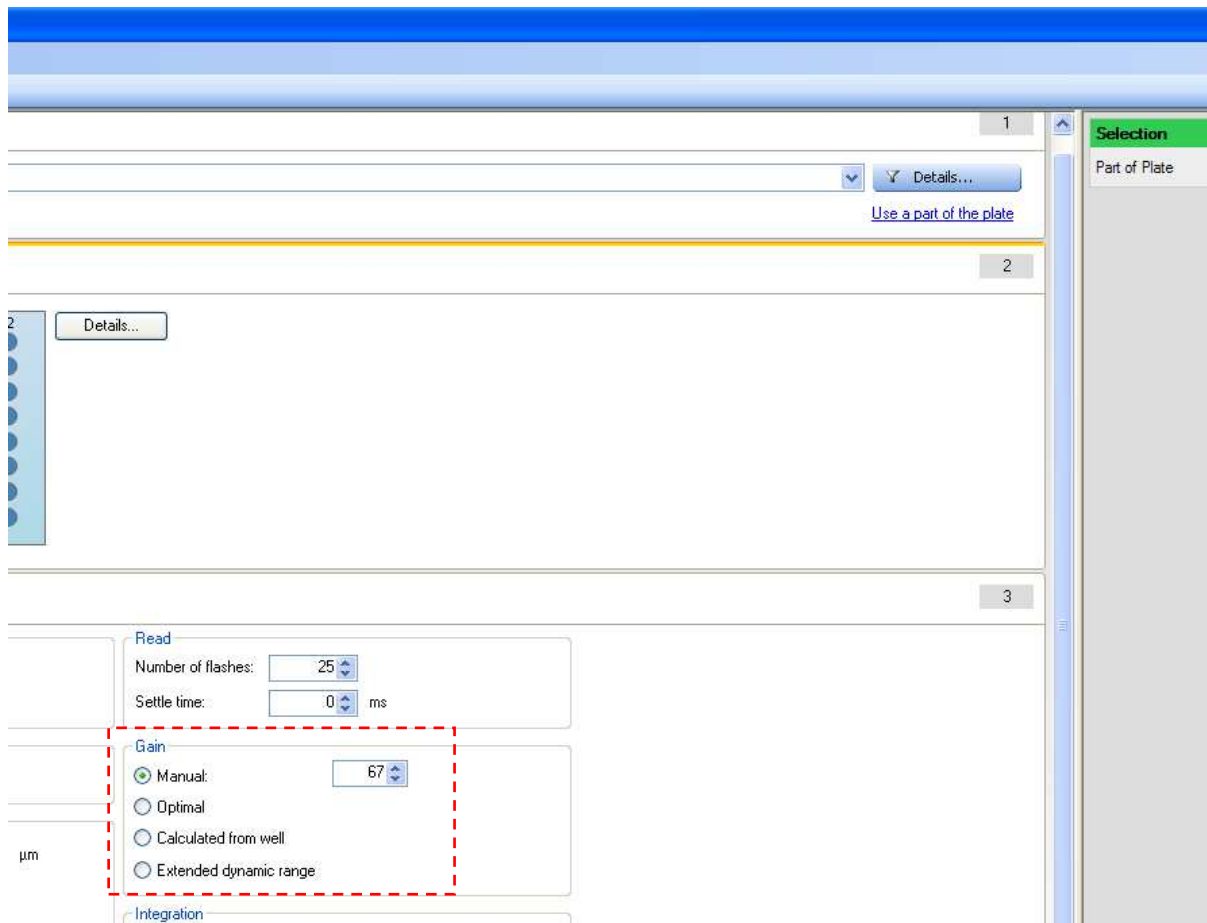


Note: Tecan Infinite® F200 (filter based) reader does not provide the Z-position adjustment. Please skip this step if using F200 Reader.

Step 4 - Gain adjustment:

Use the same plate prepared in step 3.

Select to read the well A1. Manually adjust the gain to make the reading from the positive control close to 11,000. Save or record this number. The gain setting will be valid for all Optimiser™ based assays. Readjust the gain only if a) changing the reader or b) changing the optical unit such as light bulb, filters, etc.



Technical Assistance: If you require assistance, please contact Siloam Biosciences, Inc. Technical Support at +1 (513) 429-2976 or techsupport@siloambio.com.

Additional technical assistance is available under the Technical Support tab on the Siloam Biosciences web site (<http://siloambio.com/>).

- Using Optimiser™ Immunoassay Microplate Video
- Optimiser™ User's Guide
- Reader Settings
- Quick Reference Guide
- Frequently Asked Questions
- Application Notes

Two additional videos appear under the Technology tab of the web site.

- Optimiser™ Principles of Operation
- Running an Assay with Optimiser™



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