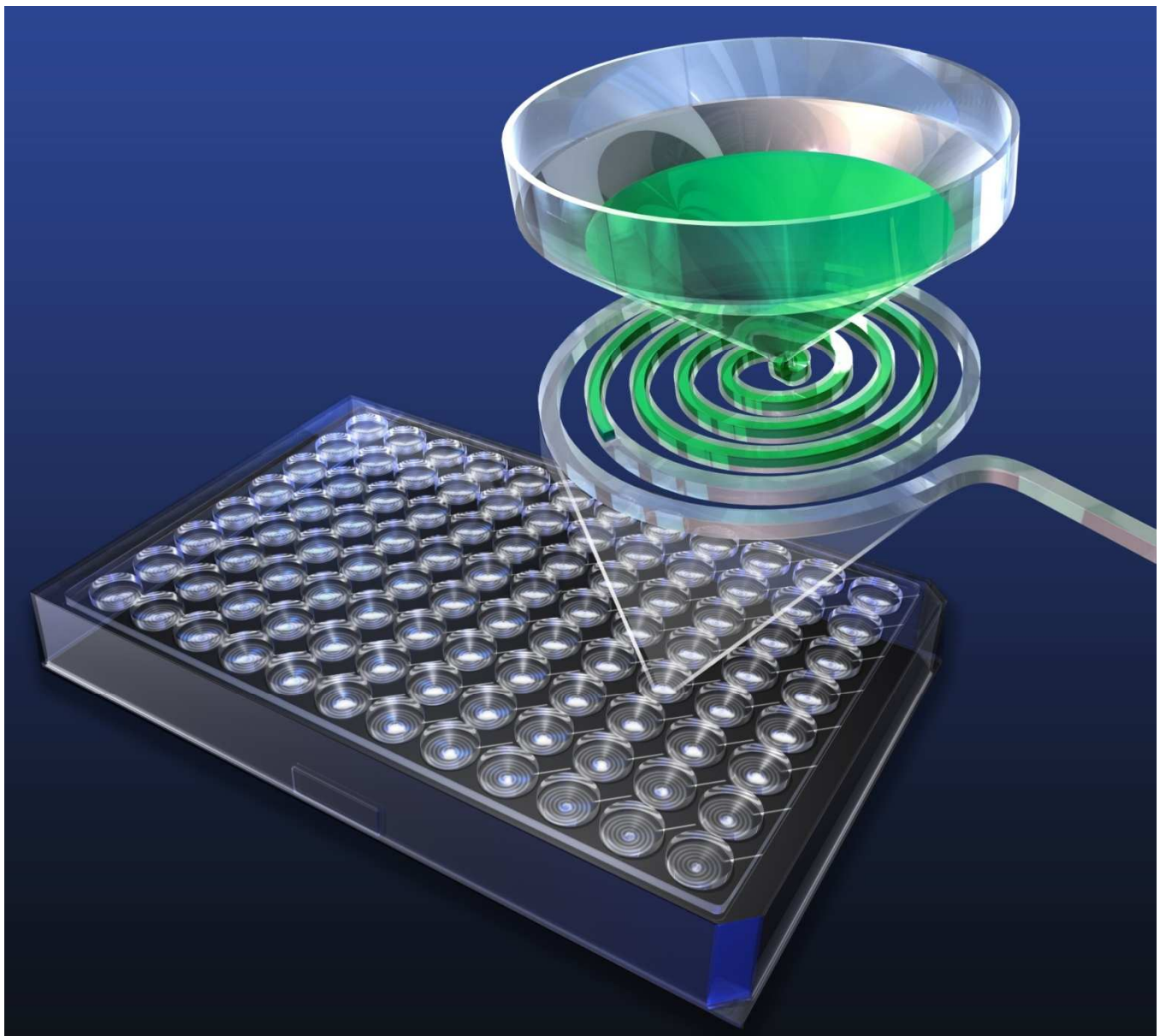


TECHNICAL NOTE

TN0001: Optimiser™ Microplate System (ELISA) General Reader Setup Guide



*Better Immunoassays through
Innovative Microfluidics*

GENERAL READER SETUP GUIDANCE:

Optimiser™ based assays are compatible with standard fluorescence plate readers and multi-mode plate readers with fluorescence reading capability. Below is the general guidance for setting up the readers. For further assistance, please contact Siloam's technical support.

Step 1: Selecting the wavelength for excitation and emission light:

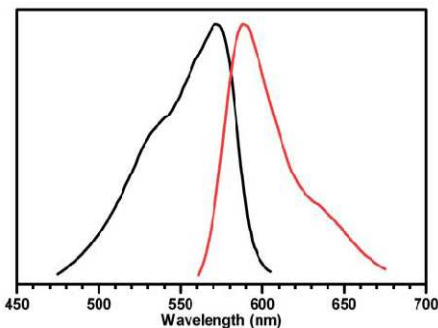


Figure 1. Normalized absorption (left) and emission (right) spectra of OptiGlow™ chemifluorescent substrate.

Assays on Optimiser™ uses OptiGlow™ substrate, which can be detected using the appropriate excitation and emission settings (Figure 1). Quantitation does not require filters that precisely match the excitation/emission maxima. However, a non-overlapping filter set with a band pass that includes the excitation/emission spectra is required. Wavelengths at 530-575 nm for excitation and 585-630 nm for emission can be used for detection. Below are examples for different types of readers:

- **Filter-based readers:** install 528/20 nm (or similar) filter for excitation, and 590/35 nm (or similar) filter for emission
- **Monochromator-based readers:** in wavelength setting, set excitation at 528 nm, and emission at 590 nm
- **Readers with pre-configured optical set:** select the wavelength setting for Rhodamine or Cy3.

Step 2: Selecting the plate type:

Optimiser™ microplate fits 96-well SBS standard in all specifications. Please use “96-well standard” or similar in plate type setting.

Step 3: Selecting the probe direction:

Please use “top reading” for probe direction.

Step 4: Selecting the sensitivity/gain:

When defining reading parameters for fluorescence analysis, setting the PMT sensitivity (or “gain” in some types of fluorescence reader) is important for obtaining useful measurements. If available, a manual sensitivity/gain setting is recommended for reading Optimiser™ microplates. The procedure is as described below:

- 1) In a clean plastic tube, add 50 μ L of OptiGlow™ A, 50 μ L of OptiGlow™ B, 1 μ L of OptiGlow™ C, and 1 μ L of Siloam's SAV-HRP stock solution, mix well, and wait for 2 minutes. The substrate will be fully developed and stable for hours. *Note: Any HRP conjugate with concentration greater than 1 μ g/mL can be used for this test with following experimental protocol*
- 2) Load 4 μ L of mixture into one well of Optimiser™ microplate and wait until the well is empty (do not use pad/holder)
- 3) Read that well in reader with various gain setting.
- 4) Select the gain which gives the RFU reading closest to 11,000.
- 5) Use the same gain setting, read one blank well of Optimiser™, the readout should be less than 50.
- 6) Save or record this gain setting.
- 7) This defines the max reading (**RFU_{max}**) that Optimiser™ based assays can reach with this reader gain/sensitivity setting.

The gain setting will be valid for all Optimiser™ based assays. Repeat Step 4 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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