User Manual

Optimiser™ Microplate System

For immunoassay (ELISA)

Catalogue Numbers: OPH-2, OPH-10, OPH-50; OP-2, OP-10, OP-50

Manufactured by:

Siloam Biosciences, Inc.
413 Northland Blvd.
Cincinnati, Ohio 45240

FOR LABORATORY USE ONLY

Read the User Manual in its entirety before using the Optimiser™ Microplate System

Intended Use:

Optimiser™ microplates are warranted to perform in conformance with published product specifications in effect at the time of sale as set forth in product documentation and/or package inserts. Products are supplied for Laboratory Use Only. The warranty provided herein is valid only when used by properly trained individuals and is limited to six months from the date of shipment and does not extend to anyone other than the original purchaser. No other warranties express or implied, are granted, including without limitation, implied warranties of merchantability, fitness for any particular purpose, or non-infringement. Buyers' exclusive remedy for non-conforming product during the warranty period is limited to replacement of or refund for the non-conforming product.

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Symbol indicates mandatory step required to ensure proper operation

Symbol indicates helpful tips to achieve optimal performance

INTRODUCTION:

Siloam Biosciences' Optimiser™ technology offers a rapid and sensitive chemifluorescent-based ELISA procedure that uses very small sample volumes. The speed, sensitivity, and small sample requirements are enabled by the unique microfluidic design of the Optimiser™ microplate. Standard immunoassay reactions such as analyte capture and detection occur within a ~ 5 µL microfluidic reaction chamber. The unique microchannel geometry and small reaction volumes favor rapid reaction kinetics. The typical assay procedure utilizes a 5 µL sample and each reaction step is completed in 10 - 20 minutes. With wash time, substrate incubation time, and read time accounted for, a typical assay can be completed within approximately 2 hours.

Please refer to the Optimiser™ Technology page on Siloam's website for more details on the principles behind the Optimiser™ microplate platform.

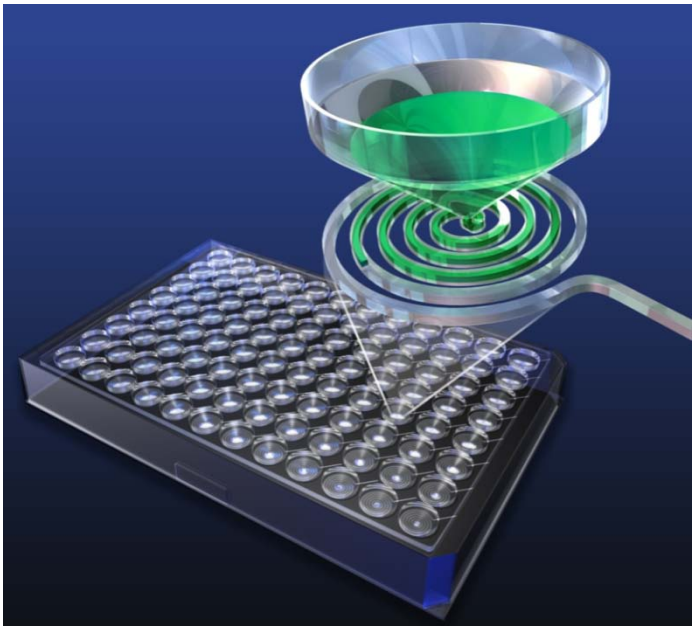

Figure 1. Optimiser™ microplate:

The Optimiser™ microplate is a revolutionary new microplate format. With an ANSI/SBS compliant 96-well layout, the Optimiser™ integrates the **Power of Microfluidics** to allow for low volume, rapid, and sensitive immunoassay protocols. Figure 1 shows the Optimiser™ microplate schematic with magnified view of one "cell" of the Optimiser™. Each cell of the Optimiser™ has a loading well (only used to add reagents) and a microfluidic reaction chamber. Reagents/samples are added to the well and transported via capillary action to an absorbent pad (not shown). The unique design of the Optimiser™ allows the well to be drained but each liquid is trapped in the channel by capillary forces. As the next liquid volume is added, the capillary barrier is broken and the liquid within the microchannel is drawn out by the absorbent pad and replaced by the new reagent. All assay reactions occur within the microfluidic reaction chamber.

UNIQUE CONSIDERATIONS FOR OPTIMISER™ MICROPLATE

Optimiser™ Microplate and Assembly:

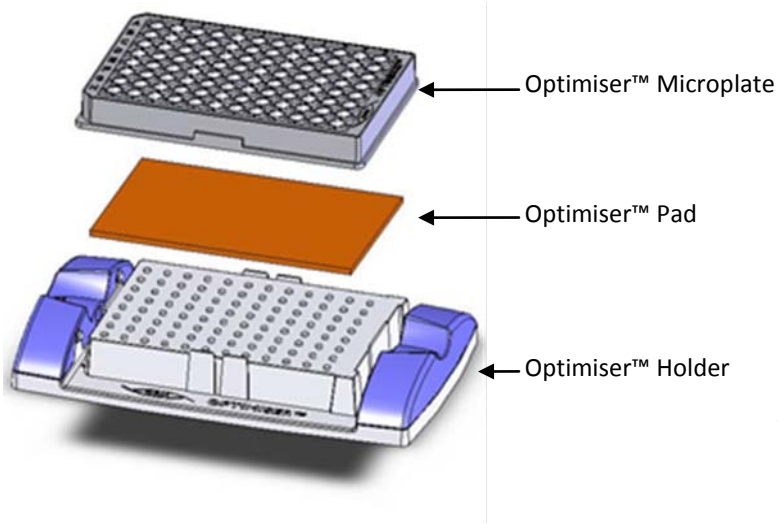

Figure 2. Optimiser™ microplate assembly
Position absorbent pad on holder, align the Optimiser™ microplate and press down gently to click-lock the plate in holder

The pad must be oriented correctly with the smooth surface (tape side) facing the holder and absorbent surface touching the microplate

Optimiser™ Microplate Pipetting Instruction:

Tutorial 1 provides hands-on training for first time users to practice pipetting with Optimiser™. Please read the entire Pipetting Instruction section before attempting Tutorial 1.

Avoiding Bubbles While Pipetting:

1. Bubbles will compromise the performance of assays on Optimiser™ by interfering with the flow of liquid within the microchannels.
2. OptiBlock™ reagent may form bubbles readily with standard pipetting techniques.
3. To avoid complications due to bubbles, Siloam Biosciences recommends the use of the "Reverse Pipetting" technique during all pipetting steps.
    a. To aspirate liquid, press the operating button of the pipette to the second stop (refer to illustration below).
    b. Immerse the pipette tip in the liquid to a depth of about 2 mm and steadily release the operating button completely.
    c. Withdraw the tip from the liquid, touching it against the edge of the reservoir to remove excess liquid.
    d. Dispense the liquid into the loading well of Optimiser™ microplate by gently and steadily pressing the pipette's operating button to the first stop. Briefly hold the operating button in this position.
    e. With the button in this position, move the tip from the loading well to the reagent reservoir, immerse the tip in the liquid and aspirate.

	Pipetting step			
Ready position	1	2	3	4
First stop	↓	↑	↓	↑
Second Stop	↓	↑		

Figure 3. Reverse Pipetting procedure

THE USE OF PROPER PIPETTING TECHNIQUE IS CRITICAL TO AVOID AIR-BUBBLES.
Air bubbles will occlude the microfluidic channel and stop the flow of the Optimiser™.

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Accurate and Precise Delivery of 5 µL Volumes:

Assays on Optimiser™ require the accurate and precise delivery of 5 µL volumes. The following guidance is offered to users.

1. Use pipette for which the upper limit of their operating range is ≤10 µL.
2. Use pipette tips appropriate for 5 µL pipetting.
3. To aspirate liquid, hold the pipette near vertical and immerse the pipette tip in the liquid to a depth of approximately 2 mm in the liquid. Withdraw the operating button steadily. Wait ~ 1 second. Withdraw the tip from the liquid.
4. To dispense liquid, hold the pipette nearly vertical. With the pipette tips **touching the surface of the Optimiser™ well**, depress the operating button steadily until the liquid is dispensed.
5. **Note:** The pipette tip must make contact with the well surface for proper dispensing (see "RIGHT" frame below). Do not pipet directly into the hole at the bottom of the well (see "WRONG" frame immediately below).

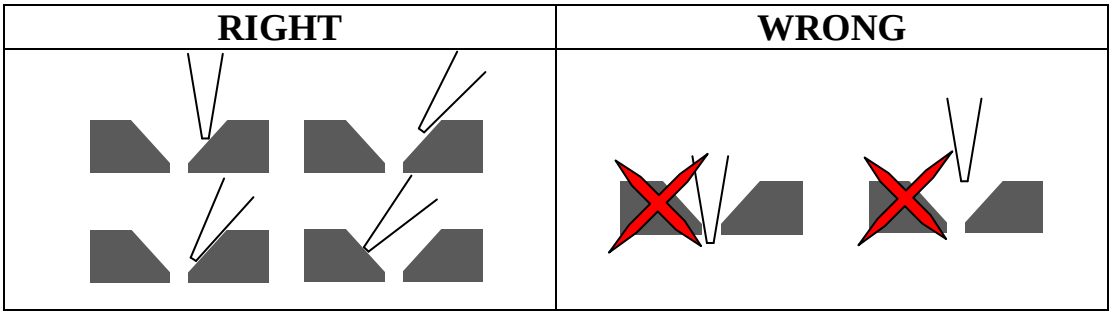

Figure 4. Pipette tip positioning for dispensing in the Optimiser™

Additional Technical Considerations:

1. The Optimiser™ system has been qualified with aqueous liquids only. Do not use solvent-containing samples.
2. The buffer reagents provided with the assay kit have been developed and validated for the Optimiser™ microplate. **Do not substitute alternate buffers or reagents.**
3. The presence of particulates in liquids dispensed to Optimiser™ wells may block liquid flow through the microchannels.
    a. Centrifuge serum samples and serum-containing tissue culture supernates for 10 minutes at 13,000 rpm prior to testing.
4. Small flow rate variations (time to empty well) do not affect assay results.

Using Electronic Multi-channel Pipette:

An electronic multi-channel pipette is ideally suited for use with Optimiser™ microplates since (a) it *eliminates* possibility of injecting bubbles and (b) can be used for convenient repetitive loads with single aspiration step for rapid reagent transfers.

General setup for using an electronic multi-channel pipette:

- Select pipette capable of delivery 5 µL & 30 µL (e.g., with volume range of 5-120 µL).
- Choose "Reverse Pipetting" in function setting.
- Use "Multiple Dispensing" mode to transfer the solution into the Optimiser™ microplate. *For example, to transfer capture antibody solution in to a full Optimiser™ microplate, set the program for 12 times dispensing, 5 µL per dispensing. Then the pipette will automatically aspirate 60 µL of solution and dispense 5 µL volumes 12 times. Users will not need to move pipette back and forth to transfer solution.*

Multichannel pipette must be used for transferring solution into the Optimiser™ plate.

If the pipette tip is pushed inside the through-hole, the tip may cause the sealing tape at the base of the Optimiser™ to de-laminate and lead to flow failure

If the pipette tip does not touch the surface of well, the solution may stick on the pipette tip end and not dispensed into the well OR may lead to air-bubbles.

Small variations in flow rates (time to empty well) do not affect assay performance. The incubation step smoothes out any flow variation differences.

An electronic multi-channel pipette can allow for loading all reagents with a single aspiration step – Ideally suited for processing multiple Optimiser™ microplates in parallel

Almost all pipetting protocols specify users NOT to touch the well surface during pipetting. Why does the Optimiser™ user guide suggest the exact opposite?

In conventional 96-well ELISA plates, if the pipette tip touches the bottom surface of the well, it may physically disrupt some of the bound bio-molecules. In the Optimiser™ all the assay reactions occur *within the microchannel*. Hence, touching the pipette tip on the loading well of the Optimiser™ has absolutely no effect on the assay performance.

For most dispensing steps in Optimiser™ based assays, users are dispensing only 5 µl volumes. If the pipette tip does NOT touch the well surface, the dispensed well volume may "bead" and stick to the end of the tip. The well geometry of the Optimiser™ is engineered to ensure smooth filling of well/microchannel provided the liquid is dispensed steadily and directly on the well surface.

See the Optimiser™ Technology page on Siloam's website for instructional videos on pipetting techniques.

Why must all materials be transferred to the Optimiser™ plate within one minute at each step in the assay procedure?

Optimiser™ incubation steps are from 10 to 20 minutes in length. Longer time to transfer material will cause time difference between each well in incubation, which may affect the assay accuracy.

How critical is the accuracy of 5 µl dispense volume?

The Optimiser™ is designed such that the 5 µl volume represents a slight excess compared to the microchannel internal volume. Provided that the dispense volume is greater than 4.5 µl, slight (even up to 10%) dispense volume variations will not affect assay results.

Why has the recommended operating volume been changed to 5 µL? I remember seeing 10 µL as recommended volume in earlier version of the FAQ.

1) Minimizing the volume helps with improving the precision. When using the 10 µl protocol, there is higher variation in the "time to empty" for different wells on each plate. This is related to the flow rate of the microchannel and larger volume show more net effect on flow duration (and variation of the duration).
2) The new 5 µl protocol also reduces the incidences of "slow" or "stopped" flow. With proper pipetting technique and by use of the new protocol, our lab tests show that flow failure rate (well does not empty after 10 minutes) is now less than ~ 0.2%.
3) We have verified through extensive assay tests that change from 10 µl to 5 µl does not affect the assay sensitivity. This is partly owing to improvements made to the OptiMax™ buffer formulations.

READER SETUP:

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:

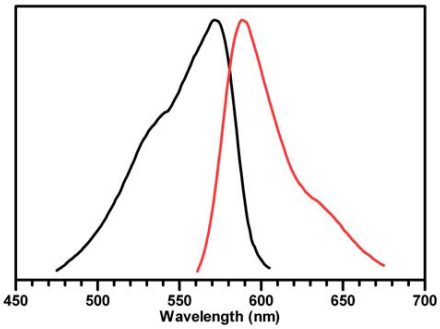

Figure 5. Normalized absorption (left) and emission (right) spectra of OptiGlow™ chemifluorescent substrate.

Assays on Optimiser™ uses OptiGlow™ substrate, which can be detected using the appropriate excitation and emission settings (Figure 5). Quantitation does not require filters that precisely match the excitation/emission maxima. However, a non-overlapping filter set with a bandpass 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 528/20 nm, and emission at 590/35 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. 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 µL of OptiGlow™ A, 50 µL of OptiGlow™ B, 1 µL of OptiGlow™ C, and 1µL of supplied SAv-HRP stock solution, mix well, and wait for 2 minutes. The substrate will be fully developed and stable for hours.
2) Load 4 µL 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.

The "Technical Support" section on Siloam's website offers detailed guidance on set up of the BioTek FLx800™ instrument as an illustrative example.

FOR FIRST TIME USER – PRACTICE WITH OPTIMAX™ STARTER KIT:

Siloam Biosciences' Optimiser™ Starter kit (CAT# OPS-IL6) is designed to provide a first-time user a comprehensive introduction the methods of use and the capabilities of the Optimiser™ platform. Specifically:

- The Pipetting Instruction Section and Tutorial 1 are designed to guide users through the correct method for pipetting to the Optimiser™ microplate. Although very similar to the conventional 96-well ELISA plate, pipetting to the Optimiser™ requires careful attention to a few key details for reliable and guaranteed performance.
- Tutorial 2 is designed to allow users to complete a model IL-6 assay. Tutorial 2 illustrates that the workflow for Optimiser™ based assays is similar but much simplified when compared to conventional 96-well ELISA plates by eliminating the traditional wash step. Tutorial 2 also shows the capabilities of the Optimiser™ to deliver equal or better sensitivity than conventional 96-well ELISA plates while using only 5 µL sample volume.

TROUBLESHOOTING:

The Optimiser™ technology and OptiMax™ ELISA kits have been designed and manufactured to ensure problem-free sample analysis. However, Siloam Biosciences has prepared the following guidance for trouble shooting problems that might be encountered due to the unique features of the Optimiser™ technology as well as problems that can be encountered with immunoassays in general.

Table 1. Trouble Shooting Guidelines

Problem	Possible Cause	Solution
Liquid does not drain from the Optimiser™ well or does not drain within 10 minutes.	A bubble is in the well.	• Disrupt the bubble with a clean 26 gauge needle. • Follow recommended pipetting guidelines. • Prepare excess reagent to avoid aspirating air. • Do not use detergents.
	Sample contains particulates.	• Centrifuge sample for 10 min at 13,000 RPM, or • Filter the sample using a 0.2 µm filter.
	Plate has lost contact with the absorbent pad or is positioned incorrectly.	• Ensure that the absorbent side (rough) of the pad is in contact with Optimiser™ and the tape side (smooth) is facing down to touch holder. • Ensure the topside of the pad is touching the bottom of Optimiser™ plate by pushing down firmly on the 4 corners of the plate. • Ensure the plate and pad are securely aligned in the holder.
No signal or unexpectedly low signal	Standard has degraded.	• Use standard on the day of its reconstitution, or • Thaw single use aliquots fresh on each test day. • Avoid repeated freeze-thaws.
	Incorrect reader filters	• Confirm filters meet requirements for substrate.
	Antibodies or SAv-HRP are degraded.	• Use within specified expiration period. • Store according to recommended storage temperature.
	Substrate was prepared incorrectly.	• Thaw OptiGlow™ - C thoroughly before preparing substrate working solution.
	Substrate working solution has degraded.	• Prepare substrate no more than 30 minutes before plate is read.
Unexpectedly high signal	Incorrect reader filters with overlapped wavelength bandwidth	• Confirm filters meet requirements for substrate.
	Reagent contamination	• Avoid cross contamination in reagents. Always change the pipet tips when handling different buffers/reagents.
Poor precision	Pipetting error (technique or equipment)	• Follow recommendations for pipetting small volumes.
Curve is nonlinear.	Pipetting	• Follow guidelines for in-plate serial two-fold dilutions.
Signal of lower standard(s) are < 0 following background subtraction.	Degraded standard	• Use standard on the day of its reconstitution, or • Thaw single use aliquots fresh on each test day. • Avoid repeated freeze-thaws.
	Degraded capture antibody	• Use within specified expiration period. • Store according to recommended storage temperature.

Technical Assistance: If you require assistance, please contact Siloam Biosciences, Inc. Technical Support at 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™

SILOAM biosciences

Better Immunoassays Through Innovative Microfluidics

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