Human IL-17A OptiMax™ ELISA Kit

Maximum Sensitivity with Minimal Sample Volume

For the quantitative determination of
human Interleukin 17A (IL-17A) in cell culture supernatant

Catalog Number: IMK-601-2

Version 072511

2 x 96 well format (192 tests)

Manufactured by:

IMGENEX
www.imgenex.com

SILOAM
biosciences

FOR RESEARCH USE ONLY

Not for use in clinical diagnostic procedures.

Please read the package insert in its entirety before using
the OptiMax™ Hu IL-17A ELISA Kit.

Note: A standard fluorescence plate reader and/or multi-mode microplate reader with fluorescence reading capability, such as, BioTek Instruments' FLx800™ Fluorescence Microplate Reader is required.

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Table of Contents

I. Background 3

II. Overview 3

III. Advantages 4

IV. Standard Data 5

V. Kit Component and Storage 6

Quick Reference 7

VI. Preparation of Reagents: IL-17A OptiMax™ 8

VII. OptiMax™ Protocol 11

A. Pipetting 11

B. Assay 12

VIII. References 13

IX. Troubleshooting 14

Appendix 1: Assembly of the Optimiser™ Plate, Optimiser™ Pad, and Optimiser™ Plate Holder 15

Appendix 2: Reader Settings 16

Appendix 3: Reverse Pipetting 17

Branding and Trademarks:

The following are formulated and/or trademarked by Siloam Biosciences: Optimiser, OptiMax, OptiBind, OptiBlock, OptiGlow, OptiWash. US and Foreign patents pending. QuantaRed substrate is supplied by Thermo Fisher Scientific Inc.

Intended Use:

Optimiser™ microplates and OptiMax™ ELISA kits (Products) 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 Research Use Only (RUO).*** *The use of this product for any clinical diagnostic applications is expressly prohibited. The warranty provided herein is valid only when used by properly trained individuals 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.*

I. Background

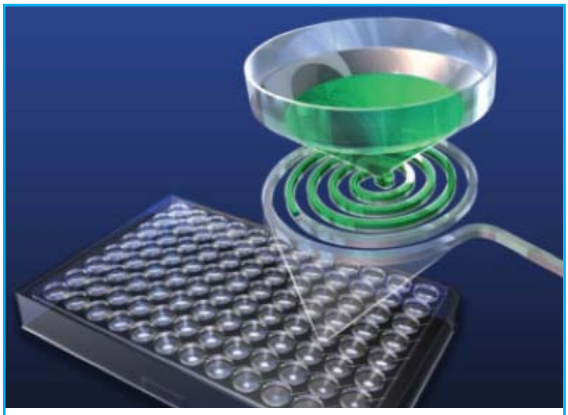
Interleukin-17A (IL-17A), a member of the IL-17 (IL-17AF) family of cytokines, is a pro-inflammatory cytokine that is produced by a subset of T helper cells that develops distinct from the Th1- and Th2-cell differentiation pathways. IL-17A, also known as CTLA-8, stimulates induction of other pro-inflammatory cytokines, such as, TNF alpha, IL-1 beta, IL-17A, and IL-8, leading to suggestions that IL-17A is involved in several chronic inflammatory diseases, such as, rheumatoid arthritis, psoriasis, and multiple sclerosis, etc. TGF-β (for differentiation) and IL-23 (for expansion) are required for induction and maintenance of Th17 (IL-17 producing) cells, which in turn induce the other pro-inflammatory cytokines.

II. Overview

The OptiMax™ line of ELISA Kits combine IMGENEX's quality antibodies and recombinant proteins with Siloam's revolutionary micro-fluidic ELISA plate and buffers into an assay that provides maximum sensitivity with minimal sample volume. The Human IL-17A OptiMax™ ELISA Kit offers a rapid and sensitive chemifluorescent- based ELISA procedure for human IL-17A that requires only 5 µL of sample. The speed, sensitivity, and small sample requirements are enabled by the unique microfluidic design of the Optimiser™ plate. The standard immunoassay reactions such as analyte capture and detection occur within a 5 µL microfluidic reaction chamber. The unique microchannel geometry and small reaction volume favor rapid reaction kinetics. The human IL-17A procedure utilizes a 5 µL sample and each reaction step is completed in 10-20 min. The assay can be completed within 2 hrs including wash time, substrate incubation time, and read time.

III. Advantages

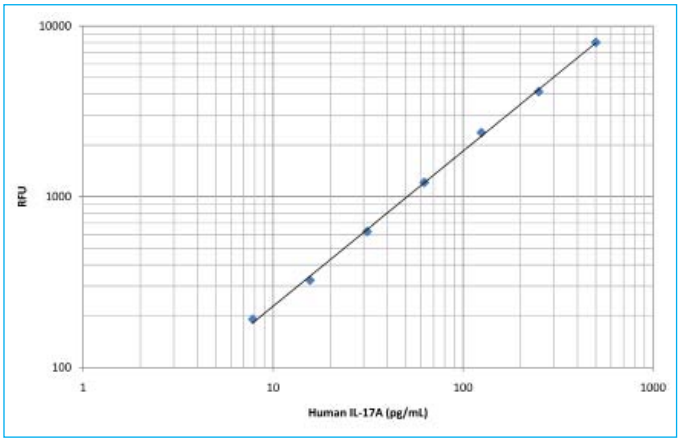
The Optimiser™ microplate is a revolutionary new microplate format. With an ANSI/SBS compliant 96-well layout, the Optimiser™ integrates the power of microfluidics to allow for low volume, rapid, and sensitive immunoassay protocols. The figure below shows the Optimiser™ microplate schematic with a magnified 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.



- True quantification with only 5 µL sample volumes.
- Multiple samples can be analyzed in a low-volume, high-throughput format.
- Full analysis complete in just 2 hrs.
- High sensitivity: detects human IL-17A at concentrations < 1.95 pg/mL.

IV. Standard Data

Following the provided protocol, the IL-17A standard was titrated to determine detectable levels.



IL-17A (pg/ml)	Average	Blank-Subtracted
500	8250	8022
250	4355	4127
125	2601	2373
62.5	1440	1211
31.2	854	626
15.6	553	325
7.8	420	192
0	228	NA

rHu IL-17A standard curve with tabulated data

Sensitivity

The Limit of Detection (LOD) [minimum detectable dose (MDD)] of IMGENEX's human IL-17A assay was determined by performing 20 replicates of assay diluent (blank) alone and calculating the mean signal + 2 standard deviations of the 20 values. The LOD is defined as the human IL-17A concentration corresponding to the mean assay blank + 2 SD. The LOD was determined to be < 1.95 pg/ml.

V. Kit Components and Storage

Table 1. Kit components and storage for the IMK-601-2 OptiMax™ Kit:

Component No.	Component	Quantity	Storage
OM601102	Capture Antibody*	70 µL	4°C
OM601202	Detecting Antibody*	25 µL	4°C
OM601302	rHu IL-17A standard	2 lyophilized vials	4°C
OM0602	SAv-HRP	25 µL	4°C
OM-052G	OptiBind™-G	10 mL	4°C
OM-055	OptiBlock™	30 mL	4°C
OM-054	OptiWash™	60 mL	4°C
OM-056	OptiGlow™-A	5 mL	4°C
OM-057	OptiGlow™-B	5 mL	4°C
OM-058	OptiGlow™-C	1 mL	4°C
OM-059	Standard Diluent	20 mL	4°C
OPT/FL-008	Optimiser™ Plate	2	RT
OPT/FL-016	Optimiser™ Holder	1	RT
OPT/FL-018	Optimiser™ Pad (Absorption of sample)	4	RT
OPT/FL-231	96-well polypropylene V-bottom plate (Dilution of sample)	1	RT
	IMK-601-2 Manual	1	RT

** This product contains 0.02% sodium azide.*

Additional items required for the ELISA test (not included in the IMK-601-2 OptiMax™ Kit)

- Microplate fluorescence reader and control software
- Analytical software
- Pipettor capable of accurately and precisely delivering 5 µL volumes

For additional lot-specific information, please refer to the accompanying Certificate of Analysis (CofA).

Quick Reference

The Quick Reference is intended as an initial overview of the ELISA procedure. Please read the entire manual before beginning the assay. Some reagents require dilution before use.

1. Assemble the Optimiser™ plate and pad in the Optimiser™ plate holder.
2. Dispense 5 µL of diluted capture antibody to the required number of wells in the Optimiser™ plate. Incubate 10 min at room temperature (RT).
3. Dispense 5 µL of OptiWash™ to the wells. Wait 10 min to proceed to the next step.
4. Dispense 5 µL of OptiBlock™ to the wells. Incubate 10 min at RT.
5. Dispense 5 µL of IL-17A standard, control, sample, and blank to the wells. Incubate 20 min at RT.
6. Dispense 5 µL of OptiWash™ to the wells. Wait 10 min to proceed to the next step.
7. Dispense 5 µL of diluted detection antibody to the wells. Incubate 10 min at RT.
8. Dispense 5 µL of OptiWash™ to the wells. Wait 10 min to proceed to the next step.
9. Dispense 5 µL of diluted SAv-HRP to the wells. Incubate 10 min at RT.
10. Dispense 30 µL of OptiWash™ to the wells. Wait 10 min to proceed to the next step.
11. Again, dispense 30 µL OptiWash™ to the wells. Wait 10 min to proceed to the next step.
12. Dispense 10 µL OptiGlow™ (A+B+C) working solution to the wells. Incubate 15 min at RT.
13. Determine the fluorescence of the wells using a microplate reader (Excitation 528/20nm, Emission 590/35nm).

VI. Preparation of Reagents: IL-17A OptiMax™`

NOTE: Bring all reagents to RT before use. Prepare all necessary dilutions (capture, detect, SAv-HRP) except for the OptiGlow™ substrate solution, which is prepared at the time of use. Dilutions can be done in sterile snap-cap tubes. Diluted antibody is then added to the V-bottom wells to be pipetted from.

OptiBind™ (Coating Buffer): OptiBind™ is provided in a ready-to-use form. No further preparation is required. Do not substitute other coating buffers for OptiBind™, as it may reduce sensitivity.

Capture Antibody: Prepare 5 µL of capture antibody working solution for each assay well to be used. Dilute the capture antibody stock 1:62.5 in OptiBind™ according to Table 2.

Table 2. Capture antibody dilution.

Number of wells to be used	Vol. of Capture Antibody	Vol. of OptiBind™	Working Solution
48 wells (half plate)	16 µL	0.5 mL	Dispense 55 µL of the working solution into each well of a single column in the polypropylene 96-well V-bottom plate
96 wells (full plate)	32 µL	1 mL	Dispense 110 µL of the working solution into each well of a single column in the polypropylene 96-well V-bottom plate.

OptiBlock™: OptiBlock™ is provided in ready-to-use form and is used to block the surface of the Optimiser™ microfluidic reaction chambers following their incubation with the capture antibody solution. OptiBlock™ is also used as the diluent for the detection antibody and SAv-HRP.

Standard Diluent: Standard Diluent is the diluent for standards, controls and samples (if sample dilution is required). It is provided ready-to-use.

Recombinant IL-17A Standard: The rHu IL-17A standard is provided in lyophilized form. Reconstitute the lyophilized standard by adding 420 µL Standard Diluent. Mix by gentle swirling until all of the lyophilized material has dissolved. Vortex gently to ensure thorough mixing of the reconstituted standard. Aliquot reconstituted standard into eppendorf tubes and store at -20°C. Avoid repeated freeze/thawing.

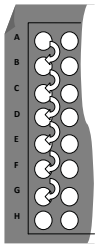
Prepare 500 pg/ml standard from stock solution using Standard Diluent. Vortex briefly to mix. *Refer to CofA for dilution instructions*.

Serial dilution of the Standard:

1. Dispense 200 µL of standard (500 pg/ml) to well A1 of 96-well polypropylene V-bottom plate.
2. Dispense 100 µL Standard Diluent to each well of B1-H1.
3. Transfer 100 µL of the standard from well A1 to B1. Change tips and mix the content of the well B1 by pipeting up and down 3-4 times.
4. Transfer 100 µL from B1 to C1. Change tips and mix the content of the well C1 by pipetting up and down 3-4 times.
5. Continue serial dilutions to G1 of the column.
6. Well H1 contains Standard Diluent which serves as blank.

Figure 1. Diluting Standard in V-bottom plate:

	Vol
A	200 µL Std 1
B	100 µL SD
C	100 µL SD
D	100 ul SD
E	100 µL SD
F	100 µL SD
G	100 µL SD
H	100 µL SD



	Conc (pg/ml)
A	500
B	250
C	125
D	62.5
E	31.25
F	15.6
G	7.8
H	0

Detection Antibody: The procedure requires 5 µL of the working detection antibody solution for each assay well to be used. Prepare a 1:250 dilution of the detection antibody stock in OptiBlock™ according to the directions in Table 3.

Table 3. Detection antibody dilution.

Number of wells to be used	Vol. of Detection Antibody	Vol. of OptiBlock™	Working Solution
48 wells (half plate)	2 µL	0.5 mL	Dispense 55 µL of the working solution into each well of a single column in the polypropylene 96-well V-bottom plate
96 wells (full plate)	4 µL	1 mL	Dispense 110 µL of the working solution into each well of a single column in the polypropylene 96-well V-bottom plate.

SAv-HRP: The procedure requires 5 µL of the working SAv-HRP solution for each assay well to be used. The SAv-HRP provided in the kit is a stock solution. The stock of SAv-HRP must be diluted with OptiBlock™ at 1:150 dilution on the day of use to make a working solution. Calculate the amount of SAv-HRP working solution required for the assay to be performed (5 µL per well + sufficient excess). The user is directed to refer to the CofA for dilution ratio.

OptiGlow™ Substrate Solution: The procedure requires 10 µL of the working substrate solution for each assay well to be used. Prepare the working substrate solution no more than 30 min before the anticipated time for reading the completed assay. For the substrate working solution, combine OptiGlow™-A, OptiGlow™-B and OptiGlow™-C in a ratio of 50:50:1 parts, respectively, according to the following table and vortex gently to mix. OptiGlow™-C may be solid at 4ºC and can be warmed in a 37º C waterbath or by slowly rotating in hand.

Table 4. OptiGlow™ Substrate solution dilution.

Number of Wells	Vol. OptiGlow™-A	Vol. OptiGlow™-B	Vol. OptiGlow™-C	Working Solution
48 wells (1/2 plate)	0.45 mL	0.45 mL	9 µL	Dispense 100 µL of the working solution into each well of a single column in the polypropylene 96-well V-bottom plate.
96 wells (full plate)	0.9 mL	0.9 mL	18 µL	Transfer the working solution into a V-shaped reagent reservoir.

For ELISA plate reader settings refer to Appendix 2.

OptiWash™: OptiWash™ is provided in ready-to-use form. No further preparation is required. The procedure requires 75 µL of OptiWash™ for each assay well to be used. Dispense OptiWash™ buffer to a V-shaped reagent reservoir according Table 5.

Table 5. OptiWash™ Working Solution

Number of Wells	Vol. of OptiWash™	Working Solution
48 (1/2 plate) (6 columns)	5 mL	Transfer 5 mL of OptiWash™ into a V-shaped reagent reservoir.
96 (full plate) (12 columns)	10 mL	Transfer 10 mL of OptiWash™ into a V-shaped reagent reservoir.

VII. OptiMax™ Protocol

Assemble the Optimiser™ Plate, Pad and Plate Holder (refer to Appendix 1).

A. Pipetting

IMGENEX recommends using the 'reverse pipetting' method to avoid adding air bubbles to the wells (an air bubble will occlude the well and prevent the well from draining).

Reverse Pipetting: To help avoid dispensing bubbles into the reaction chamber, a technique referred to as 'reverse pipetting' can be used. Completely depress the pipet's operating button to the second stop, immerse the tips in the liquid and slowly release the operating button completely. The measured volume can now be repeatedly pipetted without clearing the tips and possibly creating bubbles. (See Appendix 3 for additional information on reverse pipetting).

Accurate and Precise Delivery of 5 µL Volumes: Optimiser™ assays require the accurate and precise delivery of 5 µL volumes. The following guidance is offered to users.

1. Use a multichannel pipettor for which 5 µL is within range of accurate measurement.
2. Use pipet tips appropriate for 5 µL pipetting. These are usually referred to as '1-10 µL' tips. Larger volume pipet tips have an orifice size unsuitable for the even dispensing of very small volumes.

3. To aspirate liquid, hold the pipettor near vertically and immerse the pipet tip in the liquid to a depth of approximately 2 mm in the liquid. Release the operator button slowly steadily. Wait ~1 second. Gently touch the pipet tips against the edge of the reservoir to remove excess liquid.
4. To dispense liquid, hold the pipettor nearly vertically. With the pipet tips touching the solid surface of the Optimiser™ well, depress the operator button slowly and steadily until the liquid is dispensed.

   Note: The pipet 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).

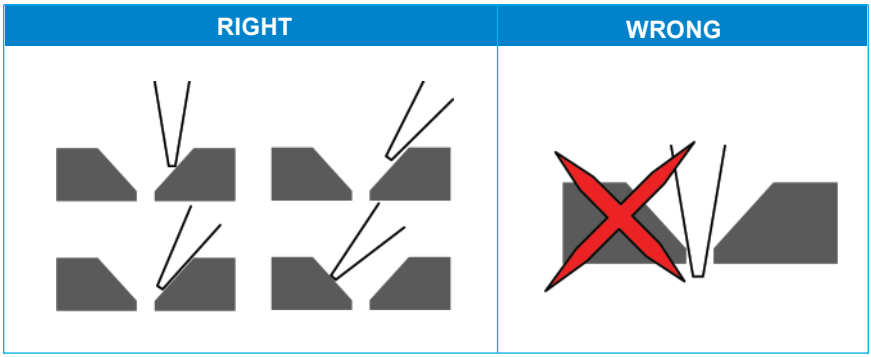


Figure 2. Pipet tip positioning for dispensing in the Optimiser™ well.

Note: Optimiser™ incubation steps are from 10 to 20 min in length. To achieve optimal assay performance, all materials must be transferred to the Optimiser™ plate within one minute at each step. To accomplish this, first place the materials to be transferred in the enclosed 96-well polypropylene V-bottom plate. Then transfer the materials to the Optimiser™ wells using a 10 µL multi-channel pipettor.

B. Assay

1. Dispense 5 µL of capture antibody solution (from the V-bottom plate, Table 2, page 8) to the required number of wells in the Optimiser™ plate. Incubate 10 min at RT.
2. Dispense 5 µL of OptiWash™ (from the V-bottom plate, Table 5, page 11) to each well. Wait 10 min to proceed to next step.
3. Dispense 5 µL of OptiBlock™ to the capture antibody-coated wells. Incubate 10 minutes at RT.

4. Dispense 5 µL of the rHu IL-17A standards (from the V-bottom plate, Figure 1, page 9), controls, samples, and blank to the required number of replicate wells of the plate. Incubate 20 min at RT.
5. Dispense 5 µL of OptiWash™ to each well. Wait 10 min to proceed to next step.
6. Dispense 5 µL of detection antibody working solution (from the V-bottom plate, Table 3, page 9) to each well. Incubate 10 min at RT.
7. Dispense 5 µL OptiWash™ to each well. Wait 10 min to proceed to next step.
8. Dispense 5 µL of SAv-HRP (see page 10) to each well. Incubate 10 min at RT.
9. Dispense 30 µL OptiWash™ to each well. Wait 10 min to proceed to next step.
10. Again dispense 30 µL OptiWash™ to each well. Incubate 10 min at RT.
11. Dispense 10 µL OptiGlow™ working solution (from the V-bottom plate, Table 4, page 10) to each well. Incubate for 15 minutes at RT.

    Caution: Observe the wells during the incubation. When the substrate has completely drained from all wells, remove the plate and pad from the holder. Discard the pad. Wipe the bottom of the plate with a Kimwipe™ to remove any liquid on the bottom surface of the plate. Step 12 will be performed at the end of the 15 min substrate incubation time.
12. Place the plate in the reading chamber of a fluorescence microplate reader. Promptly read the plate at the conclusion of the 15 min incubation. Excitation 528/20 nm (or similar) Emission 590/35 nm (or similar). For reader settings, please refer to Appendix 2.

VIII. References

1. Fielding CA, McLoughlin RM, McLeod L, Colmont CS, Najdovska M, Grail D, Ernst M, Jones SA, Topley N, Jenkins BJ. **IL-17A regulates neutrophil trafficking during acute inflammation via STAT3.** *J Immunol.* 2008 Aug 1;181(3):2189-95. PMID:18641358
2. Greenhill CJ, Rose-John S, Lissilaa R, Ferlin W, Ernst M, Hertzog PJ, Mansell A, Jenkins BJ. **IL-17A trans-signaling modulates TLR4-dependent inflammatory responses via STAT3.** *J Immunol.* 2011 Jan 15;186(2):1199-208. Epub 2010 Dec 10. PMID:21148800
3. Takeda K, Kaisho T, Akira S. **Toll-like receptors.** *Annu Rev Immunol.* 2003;21:335-76. Epub 2001 Dec 19. PMID:12524386.

IX. Troubleshooting

The Optimiser™ technology and OptiMax™ ELISA kits have been designed and manufactured to ensure problem-free sample analysis. However, IMGENEX 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.

Problem	Possible Cause	Solution
1. 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 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 holder.
2. No signal or unexpectedly low signal.	Standard has degraded.	· Use standard on the day of its reconstitution, or · Thaw single use aliquot fresh on each test day. · Avoid repeated freeze/thaws of protein.
	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 temp.
	Substrate was prepared incorrectly.	· Thaw OptiGlow™ - C thoroughly before preparing substrate working solution.
	Substrate working solution has degraded.	· Prepare substrate no more than 30 min before plate is read.
3. Unexpectedly high signal.	Incorrect reader filters with overlapped wavelength bandwidth	· Confirm filters meet requirements for substrate
	Reagent contamination	· Avoid cross contamination in reagent. Always change the pipette tips when handling different buffers/reagents.
4. Poor precision	Pipetting error (technique or equipment)	· Follow recommendations for pipetting small volume.
	Well contents are not emptying uniformly or consistently.	· See #1 above.
5. Curve is nonlinear	Pipetting	· Follow guidelines for in-plate serial two-fold dilutions
6. Signal of lower standard(s) are < 0 following background subtraction	Degraded standard	· Use standard on the day of its reconstitution, or · Thaw single use aliquot fresh on each test day. · Avoid repeated freeze/thaws of protein.
	Degraded capture antibody	· Use within specified expiration period. · Store according to recommended storage temp.

Appendix 1: Assembly of the Optimiser™ Plate, Optimiser™ Pad, and Optimiser™ Plate Holder

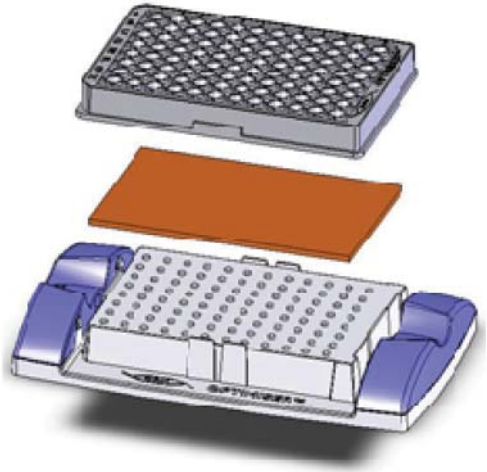
a. Place the Optimiser™ Plate Holder on the laboratory bench with the Optimiser™ logo facing the user.

b. Note that the top and bottom surfaces of the absorbent pad differ from one-another. The top side has an absorbent surface whereas a thin plastic film covers the bottom side of the pad.

c. Place the Optimiser™ Pad on the Optimiser™ Plate Holder with the bottom side of the pad facing down on the Optimiser™ Holder surface.

d. With the absorbent side of the pad on the top, place the Optimiser™ plate on top of the pad.

e. Carefully align the plate holder, pad, and plate and push the plate down firmly using thumbs and index fingers on the 4 plate corners until the plate snaps in place on the holder.



Proper Alignment of the Optimiser™ Holder, Optimiser™ Pad, and Optimiser™ Plate.

Appendix 2: ELISA Plate Reader Settings

OptiMax™ ELISA procedures are compatible with standard fluorescence plate readers and multi-mode microplate readers with fluorescence reading capability. The "Technical Support" section on Siloam's website (www.siloambio.com) offers detailed guidance on set up of the BioTek FLx800™ instrument and general guidance for other readers. Siloam Biosciences has verified the compatibility of OptiMax™ ELISA assays using OptiGlow™ chemifluorescence substrate in combination with BioTek Instruments' FLx800™ Fluorescence Microplate Reader. Siloam Biosciences uses the following wavelengths and corresponding products.

Table 3. Required Filters for BioTek FLx800™ Fluorescence Reader

Function	BioTek Part Number	Wavelength
Excitation	7082247	528/20 nm (or similar)
Emission	7082224	590/35 nm (or similar)

For the FLx800™ instrument and the filters listed above, a sensitivity setting of 45 is recommended for the reader. For more detailed information and technical support for BioTek instruments or Gen5™ software, please contact BioTek Instruments at 1-888-451-5171.

Appendix 3: Reverse Pipetting

Avoid bubbles while pipetting

1. Bubbles will compromise the performance of Optimiser™ assays by interfering with the flow of liquid within the microchannels.
2. In particular, the Standard Diluent and OptiBlock™ reagents may form bubbles readily if incorrectly pipetted.
3. To avoid complications due to bubbles, IMGENEX recommends the use of "Reverse Pipetting" techniques during all pipetting steps.

   a. To aspirate liquid, press the operating button of the pipettor to the second stop (refer to illustration below).

   b. Immerse the pipet tip in the liquid to a depth of about 2 mm and slowly 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 fluid.

   d. Dispense the liquid into receiving well by gently and steadily pressing the pipettor's operating button to the first stop. Briefly hold the button in this position.

   e. With the button in this position, immerse the tip in the liquid and aspirate.

	Pipetting Step			
Ready Position	1	2	3	4
First Stop	↓	↑	↓	↑
Second Step	↓			