AN0015: Optimiser™ Assays with Extended Dynamic Range

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Introduction:

Siloam Biosciences' Optimiser™ technology offers a rapid, sensitive, and specific chemifluorescent-based ELISA procedure using exceedingly small sample volumes. The speed, sensitivity, and small sample requirements are enabled by the unique microfluidic design of the Optimiser™ plate in which all reactions, including analyte capture and detection, occur within a ~5 µL microfluidic reaction chamber. The unique microchannel geometry and small reaction volume favor rapid reaction kinetics. A typical Optimiser™ based sandwich ELISA assay utilizes only a 5 µL sample and each reaction step is completed in 10 or 20 minutes. With wash time, substrate incubation, and read time accounted for, a typical assay can be completed within two hours.

The Optimiser™ plate is SBS/ANSI-compliant and is compatible with all standard fluorescence plate readers, robotic sample processors, and other equipment and instruments used in conducting traditional 96-well plate based assays.

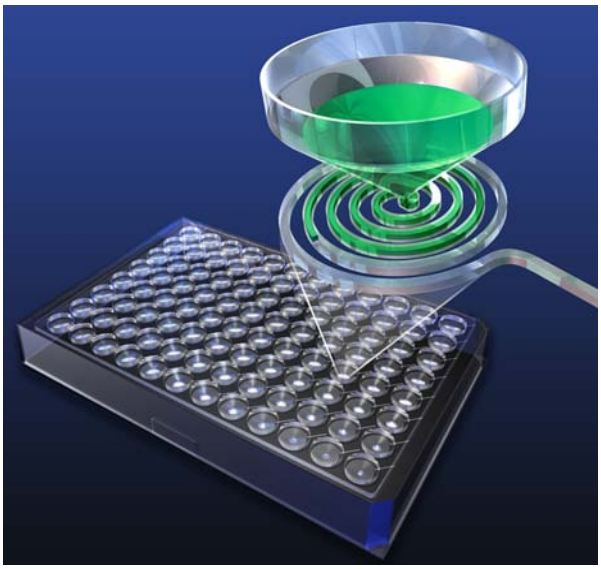


Figure 1: Optimiser™ microplate and magnified view of one "cell" of the Optimiser

ELISA Assays with Optimiser™ Technology:

The format for the most ELISAs, has remained largely unchanged for the last few decades. Typically, conventional ELISAs are performed in a series of steps each of which requires about 50-100 µL of material and 1-2 hours incubation. These steps are separated by laborious wash steps. Optimiser™-based ELISAs follow a similar reaction sequence but with notable differences

- Optimiser™ based ELISAs use only 5 µL of sample or reagent per well [20 times less per well than a conventional ELISA]. This economizes the use of valuable samples (e.g. individual mouse sera) and reagents (chemifluorescent substrates).
- Most Optimiser™ incubations are 10 minutes in length [20 minutes for samples; 15 minutes for substrate] (Figure 2).The typical Optimiser™-based sandwich ELISA is completed in about 2 hours. In contrast, the conventional sandwich ELISA requires about 6.5 hours to complete [a savings of about 4.5 hours of labor].
- Optimiser™-based ELISAs use a unique "flush" step rather than the traditional, and laborious, "wash" step used in conventional ELISAs. To flush, the user simply dispenses wash buffer into the Optimiser™ well. The wash buffer flushes the used reagent/sample from the microchannel into an absorbent pad beneath the plate. The Optimiser™ flush is equally effective as the traditional washes. The flush step also simplifies assay automation.
- The Optimiser™ **is the only platform** capable of quantifying analytes from a 5 µL sample in true ELISA format and without the need for any special liquid handling systems.
- **The dynamic range of Optimiser™ based ELISA's can easily be extended more than 10 times if needed.**

Materials and Methods:

Siloam's OptiMax™ Human IL-6 ELISA kit is used for this demonstration, which provides all of the necessary materials for testing including Optimiser™ plate, holder, and pad, and the recombinant Hu IL-6 standard, the capture and biotinylated detection antibodies, HRP-labeled Streptavidin, substrate solution, and the necessary buffers for plate coating, plate blocking, plate washing, and for diluting the assay reagents. The assay protocol on the right (Page 2, Figure 2) illustrates the typical sequence for an Optimiser™ based instructed in the User Manual of OptiMax™ Hu IL-6 ELISA kit, where a 7-point serial two-fold dilutions (64 fold total) with one zero are used for IL-6 standard.

In order to extend assay dynamic range, the exact same assay protocol is used. The only changes required are for the reagent (substrate and standard) preparation steps as described below.

1) Change the mixing ratio of OptiGlow™ substrate:

   To create the substrate working solution, combine OptiGlow™-A, OptiGlow™-B, and OptiGlow™-C in a ratio of **50:50:5.**

   Note: OptiGlow™ substrate components included in the kit contain enough OptiGlow™-C to be used for the 50:50:5 ratio. Normally, OptiGlow™ substrate is prepared by mixing Components A, B, and C in a 50:50:1 ratio respectively.

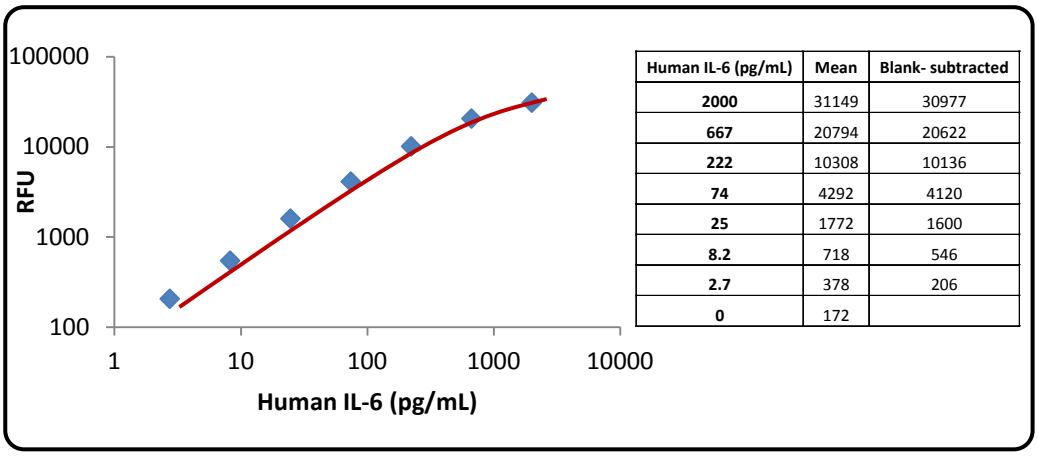
2) Using same lowest concentration (as determined by a 2-fold dilution method), use 7-point serial three-fold dilutions with one zero when making the standard. The concentrations of recombinant (r) Hu IL-6 standards are compared in the table below.

Table 1. Concentrations of rHu IL-6 standard with different dilution ratios

	IL-6 with two-fold dilution (pg/mL)	IL-6 with three-fold dilution (pg/mL)
Standard 1	150	2000
Standard 2	75	667
Standard 3	38	222
Standard 4	19	74
Standard 5	9.4	25
Standard 6	4.7	8.2
Standard 7	2.3	2.7
Blank	0	0

A standard curve is plotted on log-log scale with Hu IL-6 concentration on the x-axis and background-adjusted signal on the y-axis. A best-fit curve is drawn through the points corresponding to the mean background-adjusted signal for each standard concentration. Data in figure 3 illustrates the extended dynamic range obtained using the standard Optimiser™ assay procedure with changes described above.

Note that the results in Figure 3 are generated using the same protocol as shown on the right.



Human IL-6 (pg/mL)	Mean	Blank- subtracted
2000	31149	30977
667	20794	20622
222	10308	10136
74	4292	4120
25	1772	1600
8.2	718	546
2.7	378	206
0	172	

Figure 3. Standard curve of human IL-6 assay run in Optimiser™ with extended dynamic range.

OptiMax™ ELISA

Place Optimiser™ Plate and Pad on Holder

↓

Dispense 5 µL capture Ab

↓ 10 min RT

Flush with 5 µL OptiWash™

↓ 10 min RT

Dispense 5 µL OptiBlock™

↓ 10 min RT

Dispense 5 µL standard, sample, blank

↓ 20 min RT

Flush with 5 µL OptiWash™

↓ 10 min RT

Dispense 5 µL Detection Ab

↓ 10 min RT

Flush with 5 µL OptiWash™

↓ 10 min RT

Dispense 5 µL SAv-HRP

↓ 10 min RT

Flush 2x with 30 µL OptiWash™

↓ 2 x 10 min RT

Dispense 10 µL OptiGlow™

↓ 15 min RT

Figure 2. Typical protocol for Optimiser™-based ELISA

The efficacy of the modified substrate ratio in extending the dynamic range of assays has been verified with multiple assays as shown in Table 2.

Table 2. Typical and extended dynamic range of assays verified by Siloam

Analyte	Dynamic range with substrate component ratio of 50:50:1	Dynamic range with substrate component ratio of 50:50:5	
Human IL-6	2.3 pg/ml – 150 pg/ml	2.7 pg/ml – 2000 pg/ml	**~11 fold larger dynamic range**
Human IL-4	0.78 pg/mL – 50 pg/mL	0.82 pg/mL – 600 pg/mL	
Mouse IFN-gamma	3.3 pg/mL – 210 pg/mL	3.3 pg/mL – 2400 pg/mL	
Mouse IL-17A	2.8 pg/mL – 180 pg/mL	2.7 pg/mL – 2000 pg/mL	
Mouse IL-2	2.8 pg/mL – 180 pg/mL	2.7 pg/mL – 2000 pg/mL	

Summary:

Siloam Biosciences has successfully developed an alternate method to extend the dynamic range for all Optimiser™ based ELISA assays. Simply by using higher concentration of OptiGlow™-C in substrate working solution, the operating range of Optimiser™ based assay is now similar as chemiluminescent 96-well ELISA assay and 10 times more than colorimetric 96-well ELISA assay. Since Optimiser™ only uses ~ 10 µL substrate solution; the modified mix ratio does not add significant cost. OptiGlow™ substrate provided with all assay kits and as part of OMR series (buffer reagents) is **already right-sized** to allow use of the modified ratio.

- Siloam has also validated the Hu IL-6 assay using a **90 minute assay protocol** where each reagent incubation time is only 5 minutes. An example "90 minute assay" is described in a separate application note available in the "Technical Support → Application Notes" section of Siloam's website. The operating range of the 90 minute assay protocol is the same as the one reported here and the other assay metrics are comparable.
- Furthermore, the sensitivity of the assay can be readily "tuned" by repeating the sample addition step. Sensitivity of **femtogram/mL** is readily achievable by repeated sample loading. An example of this is described in a separate application note available in the "Technical Support → Application Notes" section of Siloam's website. Repeated sample addition (followed by 5 min incubation for each repeat) allows for a "pre-concentration" effect by allowing the surface bound capture antibodies to capture more analyte from the sample. Note that the total assay time increases slightly with each repeated sample addition step. Siloam recommends that up to 3 (no more than 5) sample addition repeats can be completed manually. If sensitivity gains beyond that achieved by 3-5 repeat loads are required, the use of a robotic sample processor is strongly recommended.

Optimiser™ Microplate, OptiMax™ buffers and OptiMax™ ELISA kits are available from Siloam Biosciences, Inc. and its distributors.

Item	Catalogue Number	Comment
OptiMax™ Evaluation Kit	OPV-IL6	Contains training material and directions for migrating a conventional ELISA to the Optimiser™ assay system
OptiMax™ Human IL-6 ELISA Kit[a]	OMA-H-IL6-02	2-plate kit
Optimiser™ microplates	OPH-02	Optimiser™ 2-pack[b]
OptiMax™ buffers	OMR-02X[c]	2-plate reagent pack[b]
Test reagent pack	OMR-TEST	To determine optimal coating buffer for a capture antibody

[a] Also available for other assay targets

[b] Also available in 10 plate and 50 plate sizes.

[c] OMR buffer packs are available with one of 12 different coat buffers. For instance, OMR-02 with coat buffer A is item OMR-02A

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Capture antibody, detection antibody, rHu IL-6 standard, and SAv-HRP provided by IMGENEX Corp under agreement. QuantaRed™ substrate is supplied by Thermo Fisher Scientific Inc.