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1.0 Introduction

The SIMplex™ 96 Multi-Array System is used to separate samples into twenty four separate chambers during incubation and wash steps on four 3" x 1" slides. SIMplex 96 eliminates broken slides, unreliable fasteners, and leaky gaskets. SIMplex 96 has been engineered to apply gentle, uniform pressure to Intuitive's ultra-thin nitrocellulose surface without leaking. Thus, SIMplex 96 maintains the integrity of your assays on ultra-thin nitrocellulose films. SIMplex 96 has been designed and validated for use with multi-channel pipets and a number of automated liquid handling instruments.

A variety of assays can be performed using Intuitive's ultra-thin nitrocellulose slides and the SIMplex 96 system, including sandwich immunoassays, capture immunoassays, protein profiling, and protein characterization. Guidelines for a typical sandwich immunoassay are provided. Modifications to the protocol to accommodate specific requirements may be necessary.

2.0 Contents

Component	Description	Size	Quantity	Product No.
SIMplex™ 96 Multi-Array Device	96-well Multiplexing Device (4 x 24 well configuration)	5.030 x 3.365 inch 85.47 x 127.76 mm	1	4-1063
SIMplex™ Gasket	Gasket compatible with 4 x 24 wells	4 x 24 Wells	1	4-1061
SIMplex™ Well Seal	Clear Polyester Adhesive Well Seal	4 x 24 Wells	25	4-1017
Low-Profile Positioning Screws	For use with automated plate washers	N/A	4	4-1031
Screwdriver	For use with the low-profile positioning screws	N/A	1	4-1032

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3.0 Materials Required, But Not Included

Component	Recommended	Description	Product No.
Microarray Slide	Protein Microarray slide	Ultra-Thin Nitrocellulose Film on a Glass Substrate (for chromogenic assays)	10-2044 10-2047
Print Buffer	5X Array Buffer	Protein Microarray Print Buffer (5X), 10 mL	2-1012
Block Buffer	5X Block Buffer	Protein Microarray Block Buffer (5X), 30 mL	2-1014
Wash Buffer	10X Wash Buffer	Protein Microarray Wash Buffer (10X), 250 mL	2-1016
Rinse Buffer	10X Rinse Buffer	Protein Microarray Rinse Buffer (10X), 250 mL	2-1018
Microarray Spotter		Compatible with standard microarray spotters.	
Automated Liquid Handling System		Compatible with standard liquid handling and automation systems.	
Microarray Scanner		For chromogenic assays, we recommend Intuitive's AthenaQuant™ System	10-1030

4.0 Storage and Stability

The SIMplex 96 Multi Array System should be stored at room temperature (20-30°C) in the original packaging until used.

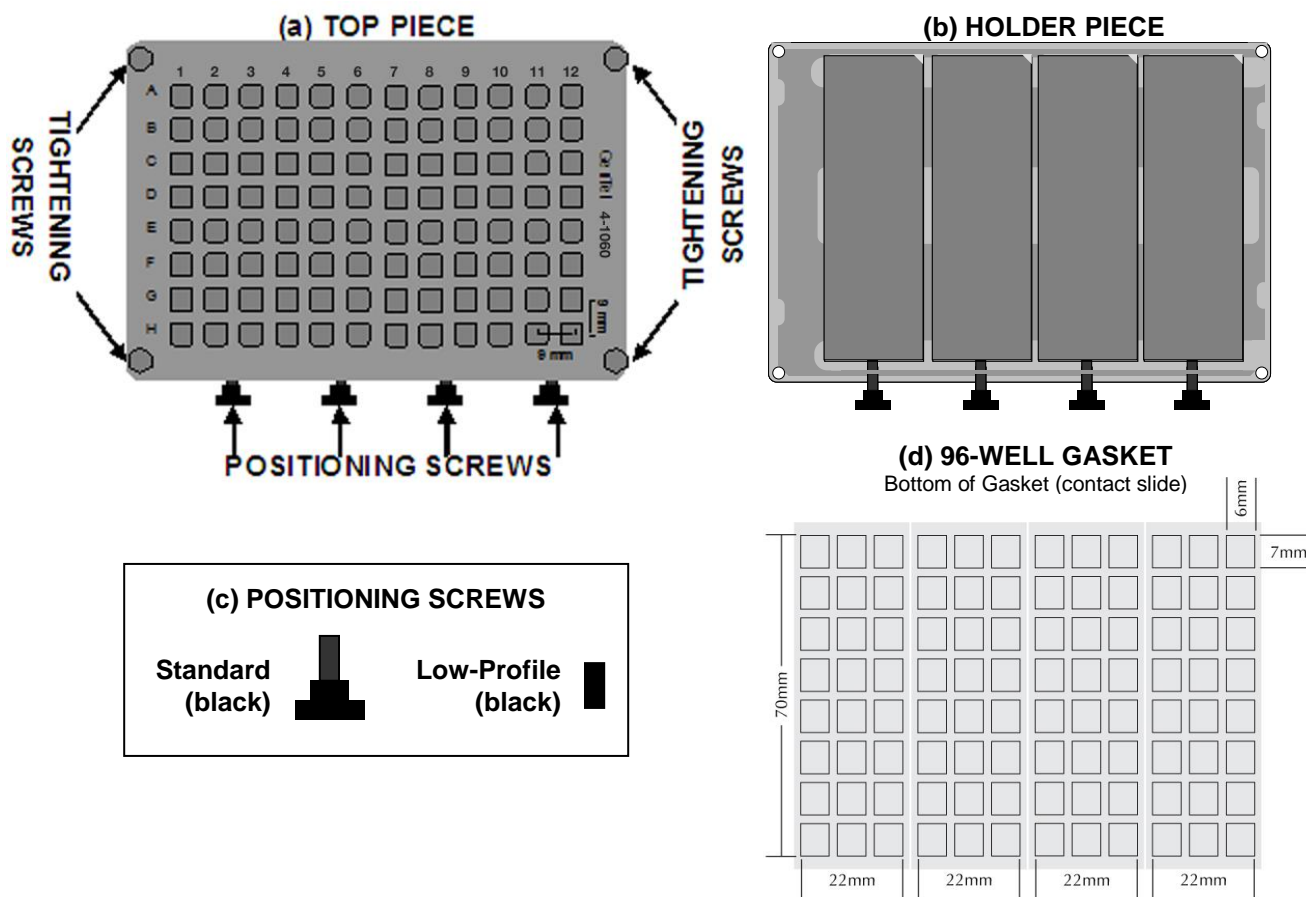
5.0 Safety and Handling

Normal precautions exercised in handling laboratory materials should be followed. The material is not considered hazardous according to 29 CFR 1910.1200. The chemical, physical, and toxicological properties of this product may not, as yet, have been thoroughly investigated. We recommend the use of gloves, lab coats, and eye protection when working with any material. The gaskets have been demonstrated to be compatible with serum and plasma.

6.0 SIMplex 96 Schematic

- 6.1 The **TOP PIECE** of the SIMplex 96 device can be easily identified by way of the scribed numbers and letters, used to locate and label each individual chamber. This is shown in Fig 1 (a).
- 6.2 The **HOLDER PIECE** of the SIMplex 96 device is shown in Fig 1 (b). SIMplex 96 comes with two types of **POSITIONING SCREWS**, shown in (c). Standard **POSITIONING SCREWS** can be used by hand but may not be compatible with automated liquid handlers. Low-profile **POSITIONING SCREWS** can be used with automated liquid handlers and require a screwdriver (included) for tightening.
- 6.3 A Schematic of the **96-WELL GASKET** is shown in (d). Gaskets have been pre-cleaned and are ready to use out-of-the package. After use, gaskets can be cleaned with detergent and/or 10% bleach solution, if required. See section 8.0 for more information on cleaning the SIMplex unit.

Figure 1 – SIMplex 96 Schematic




7.0 Methods for Sandwich Immunoassay

7.1 Array Printing

- 7.1.2 Follow directions for array printing as found in Intuitive's Protein Microarray Slide Protocol (Document No. L085).
- 7.1.3 Properly configure your microarray printer to create sub-arrays with the required spacing and alignment.

7.2 Assembly of Printed Slides in SIMplex 96

- 7.2.1 SIMplex 96 can be used with 1, 2, 3, or 4 slides. Unused positions can be left empty. If you are using automated liquid handling instrumentation, the program should be adjusted to avoid dispensing liquid into unused positions. Alternately, you can place blank slides and gaskets in unused positions.
- 7.2.2 Once the slides have been printed and are ready to be used for an assay, place the SIMplex 96 device on a clean surface so that the well openings of the device are facing up and the etched well labels are visible. Loosen all four TIGHTENING SCREWS and remove the TOP PIECE of the device.

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- 7.2.3 Loosen the POSITIONING SCREWS (shown in Fig 1(b)) on the HOLDER PIECE of the device, remove place holder slide (if present) and insert the 1-4 slides as shown in Fig 2.
- 7.2.4 If you are using an automated liquid handler, remove the Standard POSITIONING SCREWS and replace them with the low-profile positioning screws. Use of low-profile POSITIONING SCREWS requires a screwdriver (included).
- 7.2.5 Make sure the printed side of the slide is facing up. The top of the Intuitive Protein Microarray slide can be easily identified by the barcode and serial number. Place the slides in the HOLDER PIECE so the serial number faces up.
- 7.2.6 Place the gasket bottom side (thick walled) into the TOP PIECE of the device. The thin walled side of gasket will now be facing up and will contact the slide.
- 7.2.7 Gently tighten the POSITIONING SCREWS to secure the slides using your fingers. If you are using the low-profile POSITIONING SCREWS to enable use with an automated liquid handler, use the screwdriver (provided) to gently tighten.
- 7.2.8 Place the TOP PIECE of the device onto the HOLDER PIECE and gently tighten the four TIGHTENING SCREWS using your fingers.


7.3 Blocking

Note 1: SIMplex 96 has been engineered to apply gentle, uniform pressure to Intuitive’s ultra-thin nitrocellulose surface while maintaining the integrity of the ultra-thin nitrocellulose film. This enables the user to block slides after assembly in the multiplexing device using a pipettor or automated liquid handling instrumentation. Blocking is described in the Protein Microarray Slide Protocol (Document No. L085). To achieve optimal spot morphology, Intuitive recommends using our 5X Block Buffer.

For detailed instructions, follow directions for blocking as found in the Protein Microarray Slide Protocol (Document No. L085). Briefly, apply 200 µL of 1X Block solution to each well. For best results, we recommend applying blocking solution directly to the center of the array using a repeat pipettor or an automated liquid handler in a rapid, steady stream. If you are using an automated liquid handler, we recommend applying 70–100 µL of 1X Block solution directly on the array from 3 mm above the surface at a rapid flow rate. Do not let tips come in contact with the nitrocellulose surface.

- 7.3.1 Incubate for one hour with periodic agitation.
- 7.3.2 Following incubation, remove liquid from the multiplexing device by gently flicking or pipetting and discard used blocking solution. There is no need to rinse. Proceed immediately to antigen addition. Do not allow the surface to completely dry.

Note 2: If you are experiencing “comet tails” or “streaking” of spots when blocking in the wells, an alternate method is to block the entire slide by rapid immersion before assembly in the SIMplex device. To do this, fill a 50 mL conical tube with 1X Block buffer. Hold the slide approximately 1 cm above the liquid level. Drop the slide into the liquid. Immediately cap the tube and mix one or two times by inversion. It is not necessary to shake. Incubate one hour. Occasionally mix the tube over the course of incubation by brief inversion. Assemble the slide in a SIMplex 96 multi-array device and proceed immediately to antigen addition. There is no need to rinse. Do not allow the surface to completely dry.

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7.4 Antigen Addition

- 7.4.1 Apply the antigen solution to the appropriate wells (we recommend 70-100 µL). The maximum well volume is approximately 250 µL. Do not allow the surface to completely dry. Cover with the SIMplex96 Well Seal (Prod. No. 4-1017) during incubation.
- 7.4.2 Incubate according to Intuitive's Protein Microarray Slide Protocol (Document No. L085). The SIMplex 96 can be used at 37°C if required.
- 7.4.3 Following incubation, remove liquid from the multiplexing device by gently flicking or pipetting. Proceed immediately to **Step 7.5**. Do not allow the surface to completely dry.

7.5 Wash 1

- 7.5.1 Apply approximately 200 µL of 1X Wash solution to each well. Gently agitate for approximately 20 sec at RT and remove liquid from the multiplexing device by gently shaking while holding upside down. Proceed immediately to Step 7.5.2.
- 7.5.2 Repeat **Step 7.5.1** three-five additional times. After washing proceed immediately to **Step 7.6**. Do not allow the surface to completely dry.

7.4 Remaining Assay Steps

- 7.6.1 For remaining assay steps (Detector Antibody Addition, Wash 2, Detection Reagent Addition, Wash 3, Scanning), follow the Protein Microarray Slide Protocol (Document No. L085).

8.0 Care and Cleaning of the SIMplex Unit

8.1 Pre-use Cleaning


- 8.1.1 The SIMplex has been cleaned before shipping to you, but if you wish, you can wash it in soap (like Alconox®) and water. Rinse well and dry before using.

Note 3: The SIMplex gasket is soft and can be damaged by wire bristle brushes, so recommend washing the gasket by hand in soap and water. It can be sterilized in alcohol, but we would avoid exposure to harsh solvents or bleach. Always check the gasket for nicks or cuts between the wells and discard any gasket that is damaged.

8.2 Post-usage (non-biohazardous) maintenance

Cleaning after use with solutions which **do not** contain human or animal serums or plasma, cell lysates, medias or attenuated viral particles, whether concentrated or diluted (e.g. protein sample diluted in 1X Wash buffer, blocking buffer or diluents supplemented with a carrier at a concentration at or less than 10% such as bovine serum albumin, ovalbumin, mouse serum albumin, etc.

- 8.2.1 Upon completion of assay immediately rinse the SIMplex gaskets and frames with deionized water.
- 8.2.2 Submerge all components in a vessel containing soap and water.

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- 8.2.3 Using a nylon brush scrub all components (except the gasket, which should be washed by hand).
- 8.2.4 Rigorously scrub the top and bottom of each component, taking care to ensure the bristles scrub the inside of the wells of the top frame component.
- 8.2.5 Rinse with ultrapure water and allow to air dry.
- 8.2.6 Store the SIMplex in a clean location (such as a drawer) where it will stay clean.

8.3 Post-usage (biohazardous) maintenance

Cleaning after use with diluted or neat solutions containing serum, plasma or other biohazardous materials.

- 8.3.1 Upon completion of assay immediately submerge the frames and gasket in a vessel containing 70% alcohol solution or liberally spray both sides with a 70% alcohol solution.
- 8.3.2 Rinse the components with water.
- 8.3.3 Submerge all components in a vessel containing soap and water.
- 8.3.4 Soak for a minimum of 15 minutes.
- 8.3.5 Using a nylon brush scrub all components (except the gasket, which should be washed by hand).
- 8.3.6 Rigorously scrub the top and bottom of each component, taking care to ensure the bristles scrub the inside of the top frame component.
- 8.3.7 Rinse with ultrapure water and allow to air dry.
- 8.3.8 Store the SIMplex in a clean location (such as a drawer) where it will stay clean.

9.0 Ordering Information

Telephone: 608.561.8730

Toll Free in North America: 888.700.7442

E-mail: orders@intuitivebio.com

Fax: 608.826.4241

Website: www.intuitivebio.com

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