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1.0 Introduction

The SIMplex™ 24 Multi-Array System is used to divide a 3" x 1" slide into twenty four separate chambers during incubation and wash steps. SIMplex technology eliminates broken slides, unreliable fasteners, and leaky gaskets. SIMplex 24 has been engineered to apply gentle, uniform pressure to Intuitive's ultra-thin nitrocellulose surface without leakage. Thus, SIMplex 24 maintains the integrity of your assays on Intuitive's ultra-thin nitrocellulose film. SIMplex 24 wells are spaced for compatibility with a multi-channel pipettor. For multiple slide assays and compatibility with automated liquid handling instrumentation, use the SIMplex 96, which holds up to four 3" x 1" slides at the same time.

A variety of assays can be performed using Intuitive's ultra-thin nitrocellulose slides and the SIMplex 24 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	Intuitive Product No.
SIMplex™ 24 Multi-Array Device	24-well Multiplexing Device	3 x 8 Wells	1	4-1054
SIMplex™ Gasket	24-well Gasket	3 x 8 Wells	1	4-1051
SIMplex™ Well Seal, 5-pack	Clear Polyester Adhesive Well Seal	36 x 80 mm	5	4-1010
SIMplex™ Set Screw	Slide Locking Set Screw	4 mm	1	4-1033

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3.0 Materials Required, But Not Included

Component	Recommended	Description	Intuitive Prod. No.
Microarray Slide	Intuitive's Protein Microarray Slides	Transparent Ultra-thin Nitrocellulose Film on a Glass Substrate	10-2044 or 10-2047
Print Buffer	5x Array Buffer	Buffer used to print proteins on slides	2-1012
Block Buffer	5X Block Buffer	Buffer used to block non-specific binding	2-1014
Wash Buffer	10X Wash Buffer	Buffer used to wash slides between assay steps	2-1016
Rinse Buffer	10X Rinse Buffer	Buffer used to rinse slides	2-1018
Microarray Spotter		Compatible with standard microarray spotters	NA
Microarray Scanner		For chromogenic assays, we recommend Intuitive's AQ 1000 System	10-1100

4.0 Storage and Stability

The SIMplex 24 Multi-Array System should be stored at room temperature (20-30°C) in the original packaging until used. Gaskets are stable for at least 1 year from the date of purchase if stored and handled properly.

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.

6.0 SIMplex 24 Schematic

- 6.1 The **TOP PIECE** of the SIMplex 24 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 Figure 1.
- 6.2 The **HOLDER PIECE** of the SIMplex 24 device is shown in Figure 1 (b). The **HOLDER PIECE** includes a **POSITIONING SCREW**, also shown in 1 (b). The **POSITIONING SCREW** is tightened by hand (finger-tight) to secure the slide in the **HOLDER PIECE**.
- 6.3 A **24-WELL GASKET** is included. A schematic of one gasket is shown in Figure 2. Gaskets have been pre-cleaned and are ready to use out-of-the package. After use, gaskets can be maintained for extended use (see below). When assembled, the maximum well volume is approximately 250 µL.

Figure 1 – SIMplex 24 Schematic

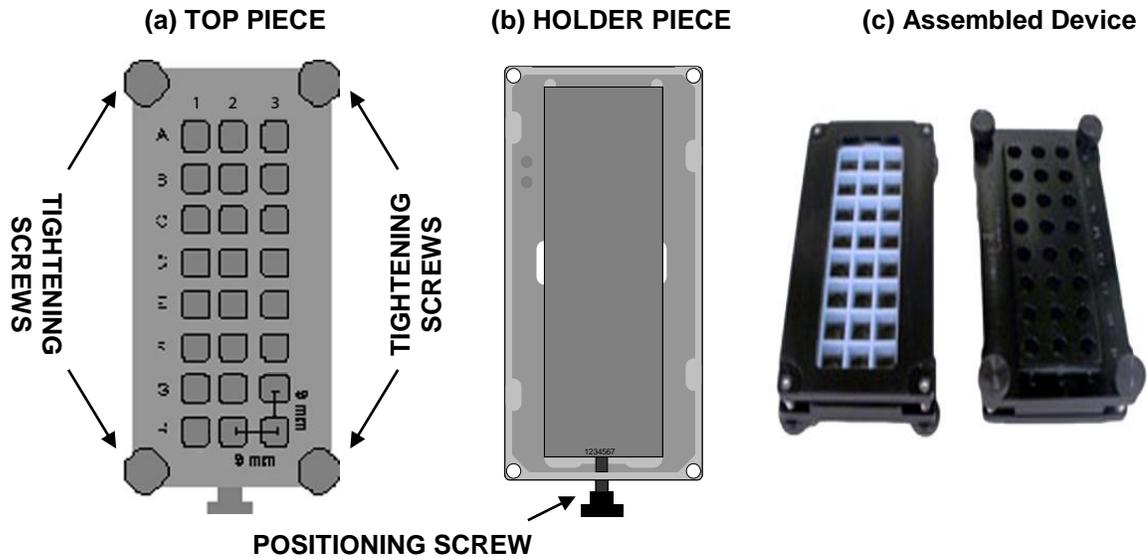
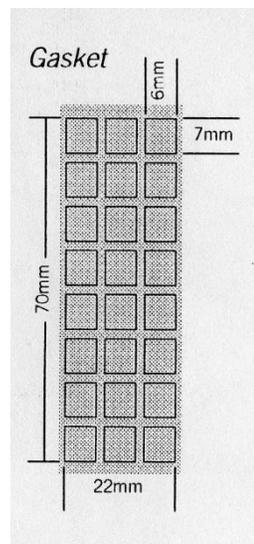


Figure 2 – SIMplex 24 Gasket Schematic



7.0 Methods for Sandwich Immunoassay

7.1 Array Printing

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

7.2 Assembly of Printed Slides in SIMplex 24

- 7.2.1 Once the slides have been printed and are ready to be used in an assay, place the SIMplex 24 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.
- 7.2.2 Loosen the POSITIONING SCREW (shown in Figure 1(b)) on the HOLDER PIECE of the device, remove place holder slide (if present), and insert your printed slide as shown in Figure 1(b). Make sure slide is facing up. The top of Intuitive ultra-thin nitrocellulose slides can be easily identified by the barcode and accompanying serial number on the active side of the slide.
- 7.2.3 Using your fingers, gently tighten the POSITIONING SCREWS **just to the point of resistance** to secure the slide.
- 7.2.4 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.
- 7.2.5 Place the TOP PIECE of the device onto the HOLDER PIECE and gently tighten the four TIGHTENING SCREWS using your fingers.

7.3 Blocking

- 7.3.1 For detailed instructions, follow directions for blocking as found in Intuitive's Protein Microarray Slide User Protocol (Document No. L085). Briefly, apply 200 μ L 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 in a rapid, steady stream. Do not let tips come in contact with the nitrocellulose surface
- 7.3.2 Incubate for one hour with periodic agitation.
- 7.3.3 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 1: 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 24 device and proceed immediately to antigen addition. There is no need to rinse. Do not allow the surface to completely dry.

7.4 Antigen/Sample Addition

- 7.4.1 Apply the antigen solution to the appropriate wells. Do not allow the surface to completely dry. Cover with the SIMplex Well seal (Product No. 4-1010).
- 7.4.2 Incubate according to the Protein Microarray Slide User Protocol (Document No. L085). The SIMplex 24 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 μ L of 1X Wash solution to each well. Gently agitate for approximately 20 sec at room temperature and remove liquid from the multiplexing device by gently tapping. Proceed immediately to Step 7.5.2.

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7.5.2 Repeat Step 7.5.1 at least 2-4 additional times. After washing proceed immediately to Step 7.6. Do not allow the surface to completely dry.

7.6 Remaining Assay Steps

7.6.1 For remaining assay steps (Detector Antibody Addition, Wash 2, Detection Reagent Addition, Wash 3, Scanning), follow Intuitive's Protein Microarray Slide User Protocol (Document No. L085). Slides must be removed from the SIMplex device for scanning.

8.0 Care and Cleaning of the SIMplex Unit

8.1 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.

- 8.1.1 Upon completion of assay immediately rinse the SIMplex gaskets and frames with deionized water.
- 8.1.2 Submerge all components in a vessel containing an excess of Alconox® detergent suspended in deionized water.
- 8.1.3 Soak for a minimum of 30 minutes.
- 8.1.4 Using a nylon brush scrub all metal components (do not use a brush on the gasket, as this may damage it over time – use a gloved hand instead to rub the soapy water into each well of the gasket).
- 8.1.5 Rigorously scrub the top and bottom of each component, taking care to ensure the bristles scrub the inside of the wells on the top frame component.
- 8.1.6 Rinse with copious amounts of deionized water.
- 8.1.7 Rinse with copious amounts of ultrapure water.

8.2 Post-usage (biohazardous) maintenance

Cleaning after use with diluted or neat solutions containing serum or plasma from human or animal sources, cell lysates, medias or attenuated viral particles.

- 8.2.1 Upon completion of assay immediately submerge the gaskets and frames in a vessel containing 70% ethanol.
- 8.2.2 Soak for a minimum 1 hour to a maximum 24 hours.
- 8.2.3 Rinse the gaskets in copious amounts of deionized water.
- 8.2.4 Submerge all components in a vessel containing an excess of Alconox detergent suspended in deionized water.
- 8.2.5 Soak for a minimum of 15 minutes.
- 8.2.6 Using a nylon brush scrub all metal components (do not use a brush on the gasket, as this may damage it over time – use a gloved hand instead to rub the soapy water into each well of the gasket).
- 8.2.7 Rigorously scrub the top and bottom of each component, taking care to ensure the bristles scrub the inside of the wells on the top frame component.
- 8.2.8 Rinse with copious amounts of deionized water.
- 8.2.9 Rinse with copious amounts of ultrapure water.

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8.3 Post cleaning maintenance

- 8.3.1 Frames
 - 8.3.1.1 Tap the frame pieces gently to remove excess liquid.
 - 8.3.1.2 Place on a paper towel to dry.
- 8.3.2 Gaskets
 - 8.3.2.1 Wrap the gaskets in a clean lint-free cloth to dry.
 - 8.3.2.2 Replace any gaskets which have abrasions, pits or roughened surfaces.

8.4 Re-use recommendations

- 8.4.1 Frames
 - 8.4.1.1 Immediately before use, rinse with copious amounts of ultrapure water.
 - 8.4.1.2 Blow dry using nitrogen gas stream.
- 8.4.2 Gaskets
 - 8.4.2.1 Immediately before use, rinse with copious amounts of ultrapure water.
 - 8.4.2.2 As the water flows over the gasket gently massage the gaskets with a clean gloved hand to remove any residual dust particles, dried salts or detergents
 - 8.4.2.3 Blow dry using nitrogen gas stream.
- 8.4.3 Immediately insert the gasket into the SIMplex frame component.
- 8.4.4 Immediately assemble the SIMplex unit with the slide.

9.0 Ordering Information

Telephone: 608.561.8730
E-mail: orders@intuitivebio.com
Website: www.IntuitiveBio.com

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