Table of Contents

1.0 Introduction

The SIM*plex*™ 64 Multi-Array System is used to separate samples into sixteen separate chambers during incubation and wash steps for four slides. SIM*plex* 64 eliminates broken slides, unreliable fasteners, and leaky gaskets. *SIMplex* 64 has been engineered to apply gentle, uniform pressure to Intuitive's ultra-thin nitrocellulose surface without leakage. Thus, SIM*plex* 64 maintains the integrity of your assays on ultra-thin nitrocellulose films. SIM*plex* 64 has been designed and validated for use with multi-channel pipettors 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* 64 system, including sandwich immunoassays, capture immunoassays, protein profiling, and protein characterization. Guidelines for a typical fluorescence-based sandwich immunoassay are provided. Modifications to the protocol to accomodate specific requirements may be necessary.

2.0 Contents

Component	Description	Size	Quantity	Part No.
SIM*plex*™ 64 Multi-Array Device	64-well Multiplexing Device	5.030 x 3.365 inch 85.47 x 127.76 mm	1	4-1029
SIM*plex*™ Gasket	16-well Gasket	2 x 8 Wells	4	4-1004
SIM*plex*™ Well Seal	Clear Polyester Adhesive Well Seal	12 x 8 Wells	25	4-1017
Low-Profile Positioning Screws	For use with automated plate washers	NA	4	4-1030
Screwdriver	For use with low-profile positioning screws	NA	1	4-1032

3.0 Materials Required, But Not Included

Component	Recommended	Description	Intuitive Prod. No.
Microarray Slide	Protein Microarray Slide	Ultra-thin Nitrocellulose Film on a Glass Substrate	10-2003 or 10-2027
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 the AthenaQuant System	10-1030

4.0 Storage and Stability

The *SIMplex* 64 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. The gaskets have been demonstrated to be compatible with serum and plasma.

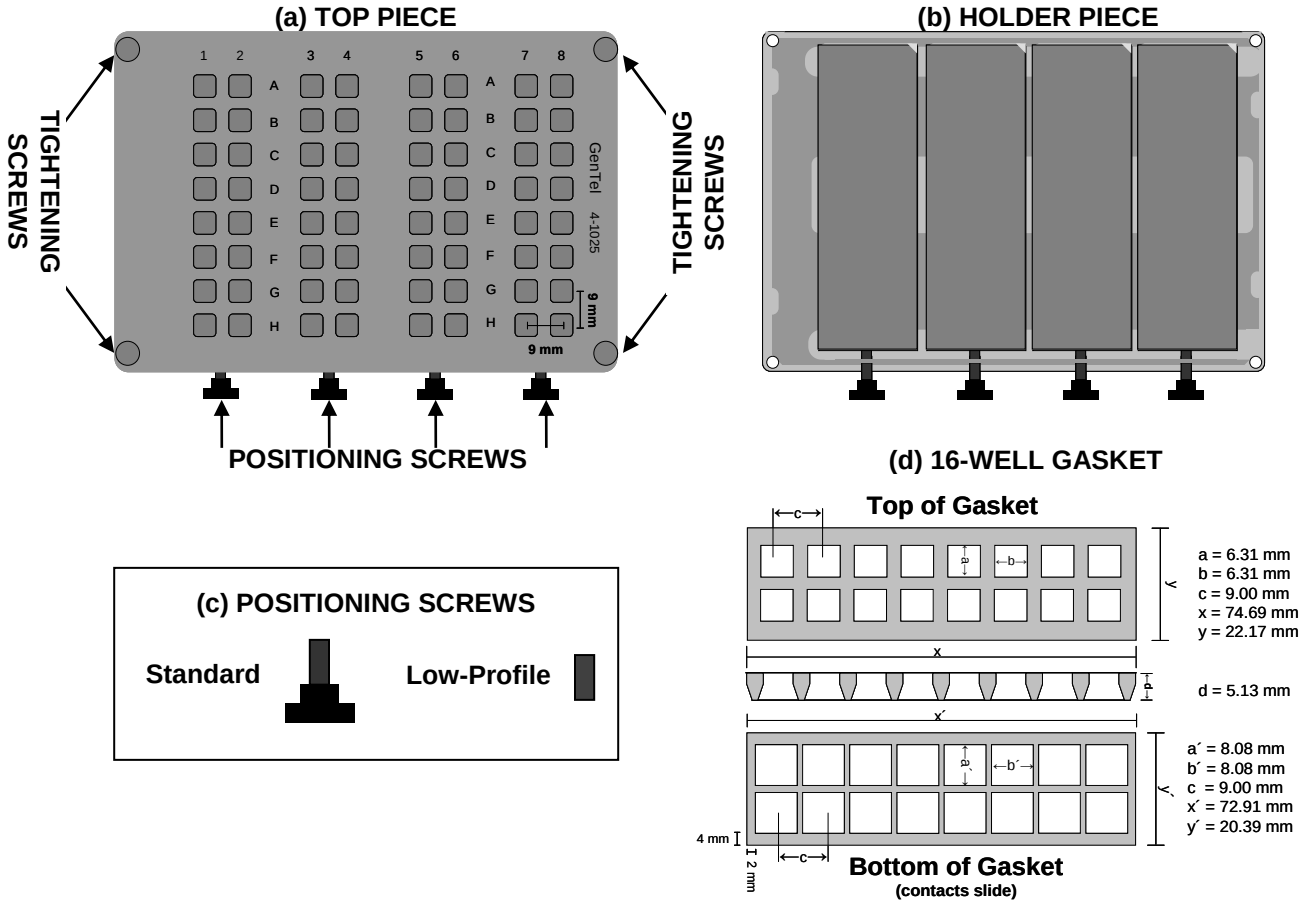
6.0 SIM*plex* 64 Schematic

6.1 The **TOP PIECE** of the *SIMplex* 64 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* 64 device is shown in Fig 1 (b). SIM*plex* 64 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 **16-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 details on cleaning protocols.

Figure 1 – SIMplex 64 Schematic

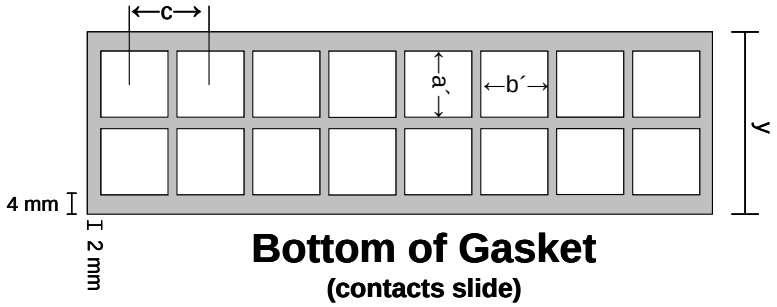


7.0 Methods for Sandwich Immunoassay

7.1 Array Printing

7.1.2 Follow directions for array printing as found in the 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. Use the dimension of the bottom of the gaskets, shown in Fig 2, below.



a´ = 6.37 mm
b´ = 6.37 mm
c = 9.00 mm
x = 74.69 mm
y = 22.17 mm

Fig 2. Schematic showing footprint of the 16-well gasket on the microarray slide.

7.2 Assembly of Printed Slides in SIM*plex* 64

7.2.1 *SIMplex* 64 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* 64 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.

7.2.3 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 1.

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 face up. The top of the Protein Microarray slide can be easily identified by the barcode and serial number. Place slide(s) in HOLDER PIECE so the serial number(s) face 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* 64 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 instrumentaion. Blocking is described in the Protein Microarray Slide Protocol (Document No. L085). To achieve optimal spot morphology, Intuitive recommends using our Block Buffer.

For detailed instructions, follow directions for blocking as found in the Intuitive 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 pipeting 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 SIM*plex* 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 SIM*plex* 64 Multi-Array device and proceed immediately to antigen addition. There is no need to rinse. Do not allow the surface to completely dry.

7.4 Antigen Addition

7.4.1 Apply the antigen solution to the appropriate wells. The maximum well volume is approximately 250 µL. Do not allow the surface to completely dry. Cover with the SIM*plex* 64 Well Seal (Prod. No. 4-1017).

7.4.2 Incubate according to Protein Microarray Slide Protocol (Document No. L085). SIM*plex* 64 can be used at 37°C if required.

7.4.3 Following incubation, remove liquid from the multiplexing device by gently flicking or pipeting. 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 room temperature and remove liquid from the multiplexing device by gently tapping. Proceed immediately to Step 7.4.2.

7.5.2 Repeat **Step 7.5.1** at least two 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 the Protein Microarray Slide Protocol (Document No. L085).

8.0 Care and Cleaning of the SIM*plex* 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 SIM*plex* 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 components.
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 gaskets and 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 components.
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 gaskets and top frame component.
8.2.8 Rinse with copious amounts of deionized water.
8.2.9 Rinse with copious amounts of ultrapure water.

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 SIM*plex* frame component.
8.4.4 Immediately assemble the SIM*plex* unit with the slide.

9.0 Ordering Information

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