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1.0 Introduction

Slide Overview

Intuitive Biosciences' Protein Microarray slides are optically clear nitrocellulose-coated slides that are designed to be used in multiplexed chromogenic immunoassays; specifically, with the SilverQuant® (SQ) Detection Kits available from Intuitive Biosciences. Nitrocellulose makes an excellent substrate for binding a wide variety of capture agents, including antibodies, proteins and other biomolecules.

Our chromogenic system includes not only slides, but also reagents, instruments and software designed for protein microarray applications. SQ reagents detect biotin-labeled molecules such as proteins that bind to protein microarrays (shown for a sandwich immunoassay in Figure 1) via a gold nanoparticle-catalyzed silver deposition. This detection scheme is designed to be flexible and can be used with a variety of different assay types; including, but not limited to, sandwich immunoassays, single capture assays, cell lysate screening, antigen arrays and serological screening. The AthenaQuant® System (a chromogenic microarray scanner and analysis software) is capable of imaging 3" x 1" slides or 96 well plates after the SQ (or other colorimetric detection) assay has been completed. To learn more about the AthenaQuant System, visit our website or call us.

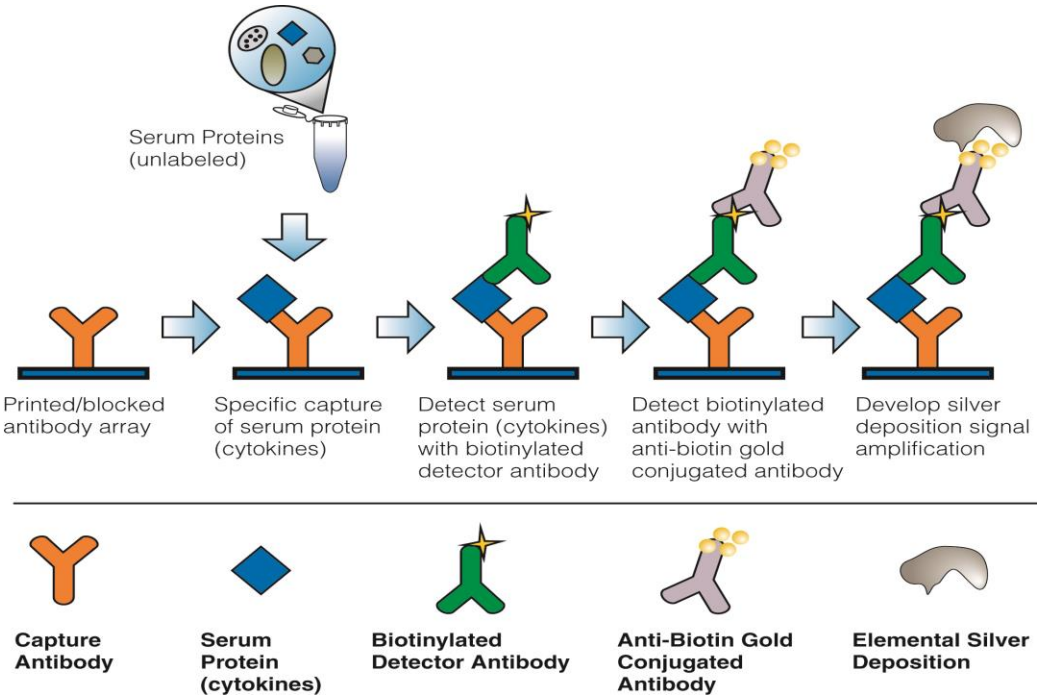

Figure 1. Schematic showing silver precipitation reaction used in the SilverQuant Detection Kit for a sandwich immunoassay for cytokines.

2.0 Kit Contents

Component	Description	Quantity	Product No.
Protein Microarray Slides	Ultra-thin Nitrocellulose coating on a transparent 3″x 1″ glass substrate	2 slides	10-2042
		4 slides	10-2044
		16 slides	10-2046
		25 slides	10-2047
Protein Microarray Slide User Protocol			L085

3.0 Materials Required, But Not Included

Component	Description /Recommended Source
Sample Separator	SIM*plex*™ 16 Multi-Array System (Product No. 4-1001), SIM*plex*™ 24 Multiplexing System (4-1050), SIM*plex*™ 64 Multiplexing System (4-1025), or SIM*plex*™ 96 Multiplexing System (4-1060).
Protein Microarray Print Buffer	5X Array Buffer (Product No. 2-1012).
Protein Microarray Block Buffer	5X Block Buffer (Product No. 2-1014).
Protein Microarray Wash Buffer	10X Wash Buffer (Product No. 2-1016).
Protein Microarray Rinse Buffer	10X Rinse Buffer (Product No. 2-1018).
Microarray Spotter	Slides are compatible with standard microarray spotters.
Chromogenic Reagents	SilverQuant Detection Kit (Product No. 10-2105) or similar.
Microarray Scanner	AthenaQuant System (Product No. 10-1030).

4.0 Storage and Stability

The Protein Microarray slides (Product No. 10-2042, 10-2044, 10-2046 or 10-2047) should be stored at room temperature (18-30°C) in the original packaging until used. If stored and handled properly, the slides are stable until the date listed on the label.

5.0 Safety and Handling

Normal precautions exercised in handling laboratory materials should be followed. The material is not considered hazardous according to 29CFR1910.1200. 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 Assay Considerations

Incubation Chambers or Gaskets are often used to keep multiple assays separate during incubation and wash steps. To best maintain the integrity of the ultra-thin nitrocellulose film, we recommend using the SIM*plex* 16 Multi-Array System (Product No. 4-1001).

7.0 Preparing to Print

7.1 **Capture Antibody Dilution.** The capture antibodies, or other biomolecules, should be diluted with an appropriate buffer for microarray printing. We suggest using our Array buffer (1X in final concentration), but other buffer formulations may also work.

NOTE: The optimal printing concentration of a capture antibody (or other biomolecule) on the slide is between 0.05-1.0 mg/mL and is typically lower than that used on a conventional (porous) nitrocellulose protein microarray slide. Optimizing capture antibody concentration to achieve the best possible spot morphology (e.g. elimination of comet tails, feathering, etc.) and signal-to-noise is recommended. A starting point for optimization of the capture antibody concentration is 0.5 mg/mL.

7.2 **Capture Antibody Printing.** Dilute samples to be printed to the desired concentration using our 5X Array buffer (or a buffer you normally print your capture reagents in) and ultrapure water. Final concentration of the Array buffer with your protein should be 1X. Proteins should be printed onto the slide at room temperature (20-30°C). The nitrocellulose coating is facing up when the barcode numbers are legible (see Figure 2).

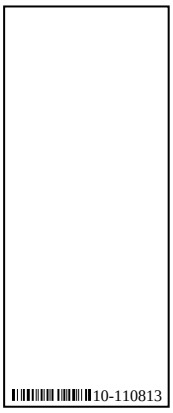

Figure 2: Drawing of the Protein Microarray slide showing the correct orientation for array printing.

7.3 Post-print Slide Incubation. The slide can be blocked immediately after printing; however, most protein types will benefit from a post-print incubation prior to blocking. The length of the post-print incubation period needed can vary from protein to protein. Ideal incubation conditions often include storage of printed slides in the dark at 18-30°C, preferably under vacuum, but under ambient pressure is also acceptable. For best results, optimize the post-print incubation period for your proteins.

8.0 Slide Blocking and Assaying

8.1 Blocking

NOTE: To best maintain the integrity of the ultra-thin nitrocellulose film, we recommend using the SIM*plex* 16 Multi-Array System (Product No. 4-1001) or SIM*plex* 24 Multiplexing System (Product No. 4-1050), or if you are testing 2-4 slides at one time, a SIM*plex* 64 Multiplexing System (Product No. 4-1025) or SIM*plex* 96 Multiplexing System (Product No. 4-1050).

8.2.1 Place the slide in a SIM*plex* (or other well-forming) device. For more information, please refer to the SIM*plex* device User Protocol.

8.2.2 If blocking the slide immediately before the assay, apply 200 µL of blocking buffer (such as our 1X Block solution) to each well. For best results, we recommend applying blocking solution directly to the center of the array in a rapid, steady stream using a repeat pipette or an automated liquid handler.

8.2.3 Incubate 1 hour at 18-30°C.

8.2.4 Following incubation, remove liquid from the multiplexing device by gently flicking or pipeting and discard used blocking solution. There is no need to rinse the slide. Proceed immediately to the next assay step. Do not allow the surface to dry completely.

NOTE: If you are experiencing "comet tails" or "streaking" of spots when blocking in the wells, blocking by rapid immersion of the entire slide is an alternate method. To do this, fill a 50 mL conical tube with 1X Block solution. Hold the SQ 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 the tube continuously, but mix the tube once or twice over the course of the one hour incubation by briefly inverting. You can dry the slide after blocking, if desired, but there is no need to rinse the slide. Assemble the slide in a SIM*plex* device and proceed immediately to the next assay step.

8.3 Assay Protocol

8.3.1 Once the slide(s) have been blocked they are ready to be assayed.

8.3.2 Antigens or Secondary Antibodies may be diluted in 1X Wash reagent, or similar buffer containing a surfactant (Tween® 20, Triton™ X-100, etc.). If desired, a carrier protein may also be added, suggested concentration is 0.1%.

8.3.3 Detection reagents should be diluted to working concentration in 1X Wash (or the buffer used in step 8.3.2).

8.3.4 If you need help adapting your assay protocol for use with the Protein Microarray slides, contact our technical support staff.

9.0 Ordering Information

Telephone:	608.561.8730
Toll-free (in N. America):	888.700.7442
FAX:	608.826.4241
E-mail:	orders@intuitivebio.com
Website:	www.intuitivebio.com