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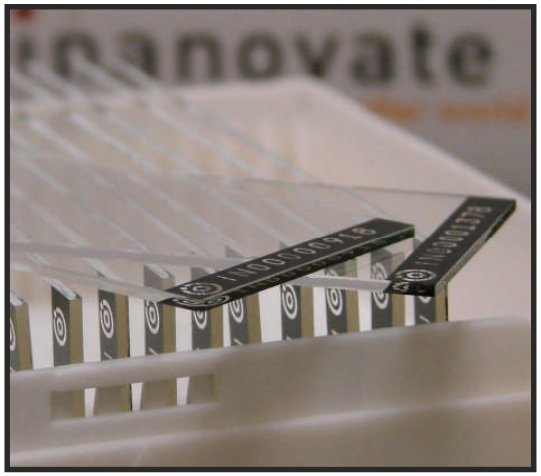
Opening doors to a smaller world



The i-Slide™ User Manual

User manual for i-Slide™ Protein Microarray Substrate

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1.0 Introduction

The i-Slide™ is the premier surface for protein microarray experiments. Its proprietary ***nano-particle enhanced surface chemistry*** helps retain protein function and control responsiveness. The i-Slide™ is ready to use straight from the box, and delivers market leading signal-to-noise, unparalleled sensitivity (down to 0.02 pg/mL, without loss of Dynamic Range), and market leading consistency (CV's as low as 2%) for protein microarray applications.

The i-Slide™ is compatible with fluorescent, chemiluminescent, colorimetric or radiographic detection systems and can be used with most microarray scanners and robots. A variety of assays can be performed using the i-Slide™, including sandwich immunoassays, reverse capture immunoassays, protein profiling, and protein characterization. Guidelines and protocols are provided herein for a typical fluorescence-based sandwich immunoassay. Additional information and protocols on various applications are available upon request.

Summary benefits of the i-Slide™

- Market leading sensitivity, consistency and dynamic range
- Simplified blocking without problems of spot spearing or comets
- Excellent long-term stability of arrayed proteins
- Compatible with all detection methodologies

- Compatible with commercially available arraying robots
- Available in easy to use format with minimal preparation requirements

i-Slide™ product codes

Product	Description	Quantity	Prod. No.
i-Slide™ Protein Microarray Substrate	Nano-particle enhanced immobilization of proteins	5	1-105
		10	1-110
		20	1-120

The **useable** surface of the i-Slide™ displays a unique ID number and logo.

2.0 Recommended Supporting Buffers and Materials

Component	Recommended	Description	Prod. No.
Print Buffer	i-Array™	Protein Microarray Print Buffer (5x), 20ml	5-110
Block Buffer	i-Block™	Protein Microarray Blocking Buffer (1x), 50 ml	5-130
Wash Buffer	i-Wash™	Protein Microarray Wash Buffer (10X), 200 ml	5-1250
Well Separator	Any available		
Microarray Printer	Compatible with all microarray printers (contact or non-contact)		
Microarray Scanner	Compatible with microarray scanners with low fluorescence background in green, blue,		

Incubation Chambers or Well Separators are often used to keep multiple assays separate during incubation and wash steps. All available well separators are compatible with the i-Slide.

3.0 Storage, Handling and Disposal

i-Slide™ substrates should be stored at or below room temperature (4-25° C) in the original re-sealable packaging. Normal precautions exercised in handling laboratory materials should be followed. 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 materials after use are waste, and should be disposed of in compliance with all currently applicable federal, state and local regulations. Such regulations change over time, and should be checked regularly.

4.0 Example Protocols for i-Slide™ Sandwich Immunoassay

4.1 General Experimental Comments

- **Sample Diluent:** The sample diluent is a critical parameter in the performance of an array immunoassay and should be selected based upon the type of samples being analyzed to maintain the assay context. The selected buffer should be used to dilute the sample, detection antibody, and labeled detection reagent.
- **Agitation:** Standard orbital shakers may produce uneven binding or swirling effects. Shakers are recommended that have a small orbital throw, agitate back and forth, and/or allow intermittent agitation.
- **Dispensing:** The use of automation or a multichannel pipette is preferred for all liquid additions (reagents and buffer) during the assay process.

4.1 Capture Antibody Printing

- Dilute antibody proteins to printing concentration in desired PRINT BUFFER. The optimal concentration of capture antibody for arraying on an i-Slide™ is 0.05-0.5 mg/ml and typically 0.4 mg/ml.
- Array antibody proteins at or below room temperature (15-21 deg C) and 30-60% relative humidity. Arrayed spots should be kept from completely drying during the printing process.
- Incubate arrayed slides at room temp in a sealed container for 8-18 hours. The original i-Slide™ container and resealable bag WITHOUT desiccant pack are suited for use a sealed container.

- Incubate arrayed slide in a low humidity container (<10% RH) for 48 hours. The original i-Slide™ container and re-sealable bag WITH desiccant pack are suited for use as a low humidity container.
- Store slides in low humidity (<10% RH) container until use.

4.2 Blocking/deactivation

- Place slide in a multi-array (16 wells/slide) device to create separated assay wells.
- Add blocking buffer solution (100uL) to the appropriate wells of the multi-array device and incubate for 30 minutes without agitation.
- Remove the majority of the liquid from the multi-array device by gently flicking the device over a waste collection tray or by using a plate washer.

4.3 Antigen/Sample Addition

- Add antigen/sample solutions (100uL) to the appropriate wells of the multi-array device.
- Cover the slide device and incubate with agitation for 2-hours.
- Remove the majority of the liquid from the multi-array device by gently flicking the device over a waste collection tray or using a plate washer.

4.4 Wash 1

- Add WASH BUFFER (100uL) to the wells of the multi-array device. Allow the slide to incubate for 10sec without agitation and remove the majority of the liquid from the multi-array device by gently flicking the device over a waste collection tray or using a plate washer.
- Repeat twice (total 3 washes).

4.5 Detector Antibody Addition

- Add detector antibody solution (100uL) to the appropriate wells of the multi-array device. The optimal concentration of the detector antibody should be between 0.2-2.0 µg/ml. Recommended starting point for optimization of the detector antibody concentration is 0.4 µg/ml.
- Cover the slide device and incubate with agitation for 1-hour at room temperature.
- Remove the majority of the liquid from the multi-array device by gently flicking the device over a waste collection tray or using a plate washer.

4.6 Wash 2

- Same as Wash 1

4.7 Labeled Detection Reagent Addition

- Add labeled detection reagent solution (100uL) to the appropriate wells of the multi-array device. The optimal concentration of the labeled detection reagent should be between 0.01-1.0 µg/ml. For DyLight 549/649 labeled streptavidin start with 0.1ug/ml.
- Cover the slide device and incubate with agitation for 30-min at room temperature.
- Remove the majority of the liquid from the multi-array device by gently flicking the device over a waste collection tray or using a plate washer

4.8 Wash 3

- Same as Wash 1&2
- Remove the slide from the multi-array device with caution to avoid touching the slide surface.
- Spin slide in a slide centrifuge at low rpm to remove excess wash buffer.

4.9 Scanning

- The dried slide can be scanned with any standard fluorescence-based microarray scanner, with the PMT set below instrument saturation for the highest intensity signal.

5.0 Buffer Use Protocols (i-Array™, i-Block™, i-Wash™)

5.1 **i-Array™ Buffer:** The i-Array™ buffer is formulated to allow the addition of specially formulated print additives during the dilution of stock proteins (capture antibodies). The buffer is 5X in print additives, but only 1X in PBS pH 7.4. For example; to make a final volume of 100uL of 100ug/ml capture antibody from a

1000ug/ml antibody stock, combine 20uL of i-Array™ buffer with 70uL of PBS pH 7.4 (or desired) and 10uL of antibody stock and mix.

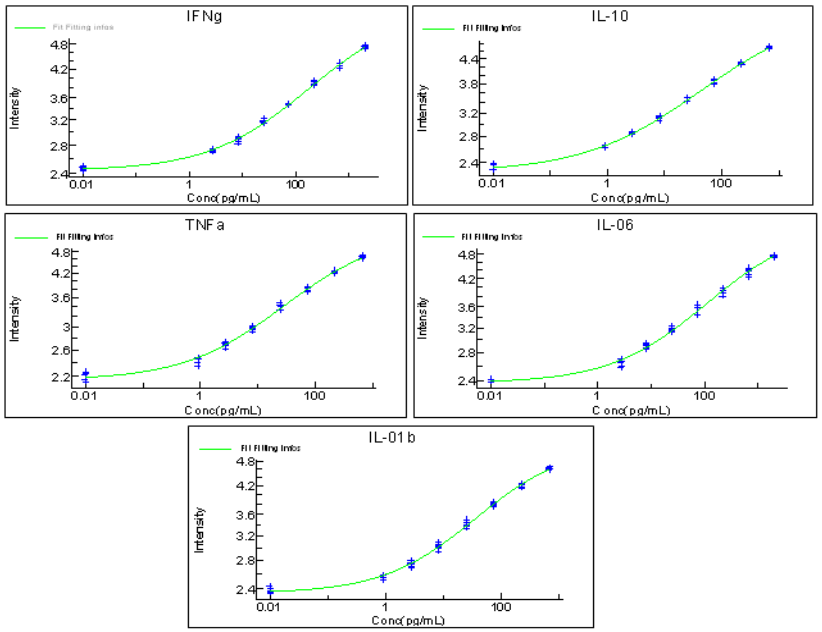
5.2 **i-Block™ Buffer:** The i-Block™ buffer is specially formulated to allow blocking just prior to performing an assay and is shipped ready to use. The buffer should be used within 4-weeks of receipt, if kept at 4 deg C. The unopened buffer may be kept frozen for 6-months or longer and used within 4-weeks after thawing.

5.3 **i-Wash™ Buffer:** The i-Wash™ buffer is formulated 10X to provide enough wash buffer for either manual or automated washing of 10 i-Slides. The buffer should be diluted 1 to 10 with deionized water prior to use.

6.0 Example Assay Data

6.1 Industry Leading Performance:

Examples of standard curves for 3 consecutive days (data points overlaid).



The standard curves illustrate the exquisite performance of the i-Slide™. These curves visually highlight the unparalleled performance metrics achievable through use of the i-Slide™; ***including sensitivities as low as 0.02 pg/mL and CV's as low as 2%.*** Specific data is included below.

6.2 Industry leading Sensitivity and Dynamic range

Limits of Detection (LOD) and Quantitation (LOQ) calculated from statistical analysis and fit to a four parameter logistic model. HLOD based on highest linear point.

Assay	LOD (pg/mL)	HLOD (pg/mL)*	LOQ (pg/mL)	Rsquared
IFNg	0.09	2000.0	2.1	0.999
IL-06	0.21	10000.0	5.8	0.997
IL-01b	0.10	10000.0	1.6	0.998
IL-10	0.02	10000.0	0.7	0.997
TNFa	0.05	10000.0	0.9	0.998

*10000pg/ml is the highest measured at 2-hours incubation time; higher limits can be obtained with a shorter incubation times.

6.3 Industry Leading Reproducibility between a) Spots, b) Wells, c) Chips, and d) Over Time:

CV measured at 3 concentrations after completion of full assay

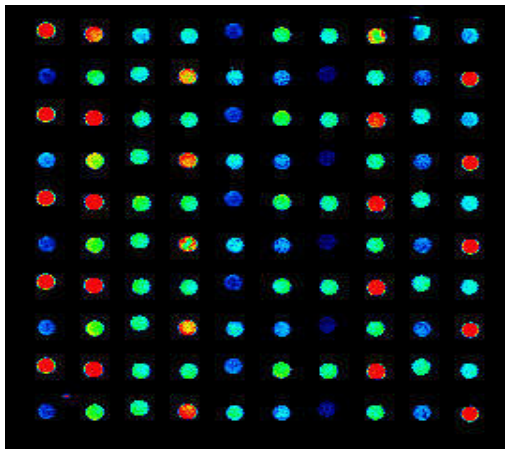
Assay	%CV Spot to Spot	%CV Well to Well	%CV Chip to Chip	% CV Day to Day
IFNg	8.4	2.0	5.2	7.4
IL-06	6.5	3.3	6.0	6.1
IL-01b	8.4	2.9	5.2	8.3
IL-10	9.2	3.4	5.2	6.8
TNFa	7.1	4.1	5.0	8.5

Example Calculations for Chip to Chip Variability (IL-01b)

Conc [pg/ml]	Mean Signal	StDev	CV
0.0	244	13	5.2
0.9	615	33	5.4
2.7	1122	19	1.7
8.2	2926	261	8.9
24.7	6695	199	3.0
74.1	17226	987	5.7
222.2	43703	2817	6.4
2000.0	57246	3800	6.6
			5.2

6.4 *High density arrays with consistent spot morphology*

Example 20 samples (5 reps each) after full ELISA assay, 10x10 array, spot spacing 500um, spot size 200um from a printed volume of 350pl using a non-contact Piezo arrayer.



6.5 *Industry Leading Recovery in Complex Matrix:*

Example serum recovery.

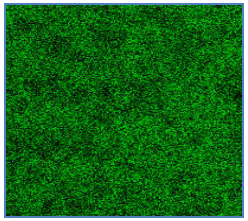
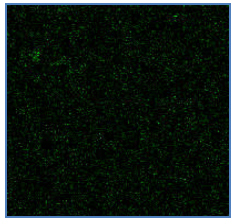
Assay	Spike1 [pg/ml]	Recovered [pg/ml]	% recovery
IFNg	222	210	95
IL-06	222	190	85
IL-01b	74	70	94
IL-10	74	76	102
TNFa	74	68	92
	Spike 2 [pg/ml]	Recovered [pg/ml]	% recovery
IFNg	74	71	106
IL-06	74	73	106
IL-01b	25	26	107
IL-10	25	27	114
TNFa	25	26	104
	Spike 3 [pg/ml]	Recovered [pg/ml]	% recovery
IFNg	25	26	96
IL-06	25	26	98
IL-01b	8	9	104
IL-10	8	9	108
TNFa	8	9	107

ICH and FDA guidelines for quantitative immunoassays stipulate a % recovery error requirement of +/- 20% for 'quantitative' classification of an assay. The above table illustrates the i-Slide's quantitative performance across a range of assays. The average % recovery error across all illustrated assays is an industry leading 6.5%, making the i-Slide™ the premier surface for quantitative protein profiling applications.

6.6 Ultra low background in Cy3 and Cy5

Images collected on ScanArray 4000 with Cy3 filters and identical settings.

The 'ultra-low' background of the i-Slide is clearly demonstrated. This unique property feeds into the i-Slides industry leading performance.

Thick nitrocellulose	Thin nitrocellulose	i-Slide
		
Mean of fluorescence 65,000	**Mean of fluorescence 1641**	**Mean of fluorescence 225**

6.7 Performance Summary

Typical performance metrics of existing leading protein microarray surfaces, across a range of common assays, are sensitivities of ~1 pg/mL and CV's of 10 to 15%. When these industry standards are compared to the performance data of the i-Slide™ (Sensitivities as low as 0.02 pg/mL and CV's as low as 2%), the advantages offered to protein resesearchers targeting higher quality data is clear.

7.0 Ordering Information

If you would like to place an order for i-Slides and/or supporting materials; please contact our enquiries team through one of the following channels:

Email: enquiries@inanovate.com
Website: www.inanovate.com
Telephone: +1 919-354-1028

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