Protocol

Nexterion® Slide NC-W 16 **Protein application**		
	Dok-Nr.:	LS6-HBM-M-002
	Version:	1.1
	Seite:	1/7
	Datum:	© April 2009

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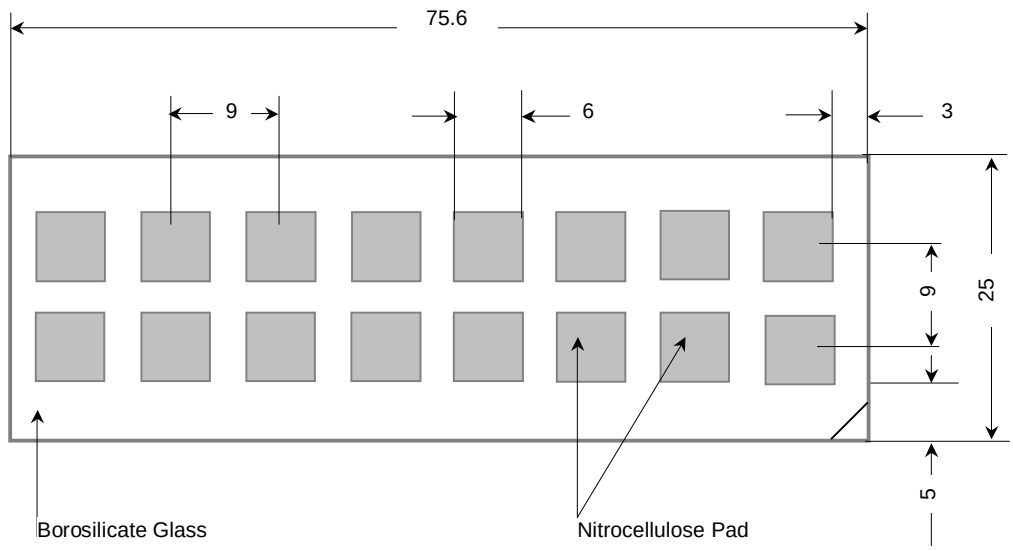
1 Introduction

PRODUCT OVERVIEW

The Nexterion® NC-W 16 slides use a nitrocellulose coating developed by Sartorius specifically for protein microarray applications. The three-dimensional structure and pore size has been optimized to offer a high binding affinity for proteins, and to better stabilize their active conformation. The SCHOTT nitrocellulose slide membranes are manufactured using a newly developed casting process, where solvents are evaporating from a mix of different cellulose nitrate polymers, and additives, to form the unique micro-porous structure. During manufacture, the thickness, and physical characteristics of the coating are tightly controlled, these together with a surface finishing process allow the reproducible manufacturing of slides with an outstanding consistency and performance.

Nexterion® Slide NC-W 16 is manufactured using the highest quality glass (standard dimensions of 75.6 mm x 25.0 mm x 1.0 mm). The slide contains 16 pads (spotting area); each 6 by 6 mm, pad centre-to-centre spacing - 9 mm and a orientation mark in the corner (see figure below).
Nexterion® Slide NC-W 16 has a high capacity for protein binding, due to its micro-porous structure and binds proteins in a non-covalent manner. The coating provides an ideal environment for proteins and enables long-term protein stability and functionality. Nexterion® NC-W 16 slides are ready-to-use from the box.



2 Storage and handling

1. The Nexterion® NC-W 16 slides are stable for over one year under ambient conditions (15 - 30 °C) and should be stored in the original boxes dry and dark.
2. Avoid direct contact with the surface of the slides to minimize contamination and abrasion of the coated surface. Always wear gloves and hold slide edge.

3 General precautions

1. The protocols contained in this document are meant to be general guidelines only and some optimization may be required depending on the application and sample being used.
2. Refer to manufacturer supplied Material Safety and Data Sheets (MSDS) for proper handling and disposal of all chemicals.
3. Nexterion® Slide NC-W 16 is for research use only, not for *in vitro* diagnostic use.

4 Reagents required

1. Protein Print Buffer: We recommend the use of our Nexterion® Spot PB (pH 7.5) (see notes about protein concentration for spotting below), alternatively other buffers can be used that provide a suitable balance between stabilizing the protein of interest and providing a reasonable print performance.
2. Blocking Solution (TBST): 150 mM NaCl, 100 mM Tris/HCl, 0.1 % Tween® 20 pH 7.5.
3. Incubation Buffer (PBST): 137 mM NaCl, 2.7 mM KCl, 4.3 mM Na_2HPO_4, 1.4 mM KH_2PO_4, pH 7.5 with 0.05 % Tween® 20.
4. Wash Buffer (PBST/PBS): 137 mM NaCl, 2.7 mM KCl, 4.3 mM Na_2HPO_4, 1.4 mM KH_2PO_4, pH 7.5 (with 0.1 % Tween® 20 for PBST only).

5 Equipment required

1. Exsiccator with a relative humidity of 10 - 25 %.
2. Centrifuge with slide holders.
3. Coplin jars (VWR 25457-006) or slide dish and rack combo (Fisher 900200) for washing slides.
4. Incubation chambers or gaskets.

6 Protein concentration for spotting

Nexterion® Slide NC-W 16 provides non-covalent attachment by the proprietary chemistry. The coupling efficiency of the non-covalent chemistry depends on a number of factors, including pH, protein print concentration, and the incubation time.
A protein probe concentration ranging from 0.05 to 1 mg/ml is recommended to ensure sufficient protein loading and to enable reliable and consistent assay results.

7 Array printing

1. Nexterion® Slide NC-W 16 is compatible with all microarray printing or spotting methods, including contact printing and piezo or ink-jet technologies.
2. Nexterion® Slide NC-W 16 is ready to use and no activation steps are required.
3. Nexterion® Slide NC-W 16 arrayed should be stored desiccated overnight at room temperature. In some cases incubation temperatures need to be adjusted to the nature of the relevant protein (e.g. 4 °C for temperature sensitive proteins).

8 Storage of printed slides

If you want to store printed arrays, please do so after printing/immobilization, but before washing/blocking. The printed protein arrays can be placed in a slide box and should be stored sealed dry and dark at room temperature (or an appropriate temp. according to the proteins printed) up to one year depending on the nature of protein.

9 Washing and blocking

As non-fat dry milk can add to the fluorescent background, we recommend to don't use in the blocking or assay steps.

1. Submerge the slides in TBST buffer for one hour under moderate agitation.
   Note that lab gloves may contain residues that can contaminate the surface and can lead to increased, non-uniform background. Avoid allowing residues from the gloves to flow onto the array.
2. Repeat step 1 for 5 min.
3. Wash the slides in diH_2O for 1min.
4. Dry the arrays by centrifugation (200 x g for 5 min).

10 Assay conditions

Note: The protocol recommended below is a general guideline; alternative steps/solutions may be required depending on the application.

The printed Nexterion® Slide NC-W 16 slides are robust and compatible with most conditions encountered in protein-based assays. However, an incubation buffer comprised of phosphate buffered saline with 0.05 % Tween® 20 (also used as Incubation Buffer, see description under Reagents Required) is recommended. It is not advised to use non-fat dry milk containing buffers.

11 Target incubation

The use of either single-use superstructures (can be ordered separately), or re-usable incubation chambers, depend on the intention of the user, is recommended for carrying out the post-printing binding reactions.

1. Dilute the labeled target in an appropriate amount of incubation buffer to allow full array coverage.
2. Apply the superstructure on the slide from top or by inverting the slide on the superstructure (figure 1), followed by firmly securing the superstructure on the slide (see figure 2).
3. Pipette the target containing incubation buffer onto the array surface, without damaging the surface (figure 3 shows 2 Nexterion® NC-W 16 slides in a SCHOTT Nexterion® MPX-4 tray for rapid processing). Use appropriate sealing strips to close the reaction chambers (figure 4). For longer incubation times humidity chambers are recommended to avoid drying of the target solution.

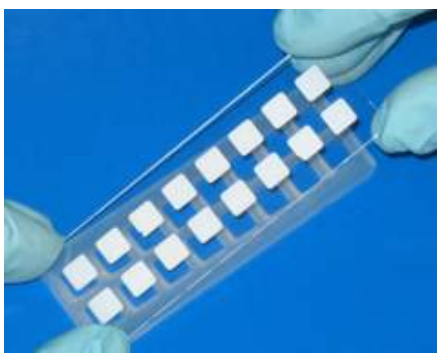


Fig.1 Application of the superstructure in inverted position

Fig. 2 Securing the superstructure

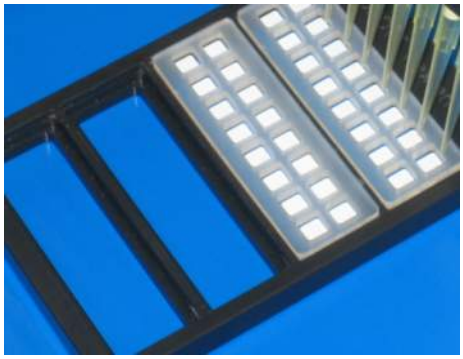


Fig. 3 Addition of target

Fig. 4 Seal reaction chambers

4. Incubate one hour to overnight (protect from light to avoid bleaching of the fluorophor) at the desired temperature under gentle agitation.

12 Washing

Caution: Do not allow slides to dry between washes, and protect from light whenever possible.

Note: The solutions recommended below for washing are a general guideline; alternative washes may be required depending on the application.

1. Remove the target solution from the slide.
2. Rinse reaction chamber(s) with PBST containing 0.1% Tween® 20.
3. Remove the superstructure carefully if needed.
4. Wash with PBST containing 0.1 % Tween® 20 with shaking for 5 minutes.
5. Repeat step 4.
6. Wash in PBS for 5 minutes with agitation.
7. Wash in diH_2O for 1 minutes with agitation.
8. Dry the array in an oil-free air or nitrogen stream or by centrifugation (200 x g for 3 - 5 min) to avoid any water stains on the slide surface.
9. Protect the array from light, dust, and handling until ready for scanning.

13 Important information about patents

Using arrays based on SCHOTT Nexterion® products for dual color analysis on a single array in which at least two different samples are labeled with at least two different labels may require a license under one of the following patents: U.S. patent nos. 5,770,358 or 5,800,992 or 6,225,625 and U.S. patent no. 5,830,645. Manufacturing and use of probe arrays may require a license under the following patents: U.S. patent nos. 6,040,138 or 5,445,934 or 5,744,305 and under the following patents owned by Oxford Gene Technology Ltd. („OGT"): European patent no. EP 0,373,203, U.S. patent nos. 5,700,637 and 6,054,270 and Japanese patent nos. 3393528 and 3386391 ("The OGT patents"). Other patents may apply. The purchase of Nexterion® products does not convey any license under any of the OGT patents or any of the other patents referred to. For all applications SCHOTT North America Inc. and SCHOTT Technical Glass Solutions GmbH make no representation or warranty that the practice of its technology and products or any improvement will not infringe or violate any domestic or foreign patent of any third party. Before making or using any oligonucleotide arrays you should contact OGT to discuss a licence. To inquire about licensing under the OGT patents, please contact OGT at licensing@ogt.co.uk.

14 Compatible Accessories

Item	SCHOTT Product	Grace Biolabs*	Whatman**
Nexterion® Slide NC-W Single pad slide	-	SecureSeal* Hybridisation chambers Internal 53 x 22 mm Order code: SA2657	Single-Well Incubation Chamber Order code: 10486137
Nexterion® Slide NC-W 16-pad slide	-	1. ProPlate* slide module Order code: 204862 2. SecureSeal* Hybridisation chambers 16 chambers, 7 x 7 mm, 9 mm spacing Order code: SA 16S-0.5	16-Well Array Incubation Chamber Order code: 10486046
Holder for processing single slides	-	-	Chip Clip** Slide-Holder Order code: 10486081
SBS compliant microplate holder for processing 4 slides	**Nexterion® MPX-4 tray Reusable** **Order code: 6045408**	FlexWells* slide tray Order code: 204970	FAST** Frame Multi-Slide Plate Order code: 10486001

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