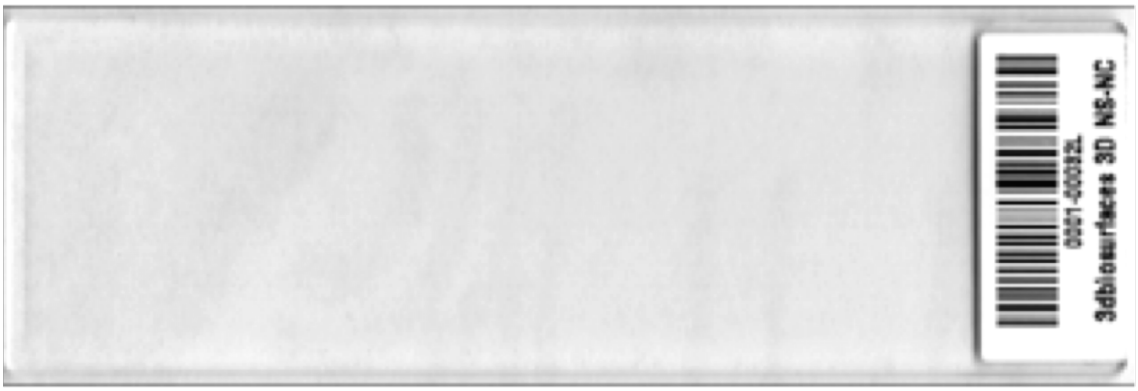
3dbiosurfaces

PROTOCOL FOR 3dbiosurfaces® 3D NS-NC SLIDE

PROTEIN APPLICATIONS

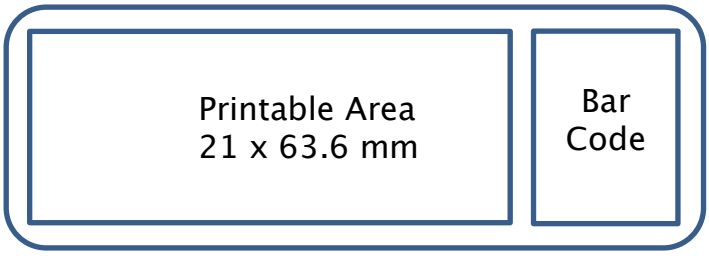


1) Introduction

3dbiosurfaces® 3D NS-NC slide is manufactured to standard dimensions of 75.6 mm x 25.0 mm x 1.1 mm. (The dimensions can vary by +/- 0.25mm, 0.2 mm and 0.05 mm, respectively).

All slides are bar coded with nanostructured surfaces (NS) on the same side as the bar code label.

The nanostructured surfaces begin 0.2 mm from the edge of the top and sides of the slide, and above the bar code label.



The uncoated (NC) slide surface allows for the efficient binding of molecules, such as proteins. The surface of the slide is hydrophobic. We recommend UV treatment of the slide before printing in order to improve the wettability and aid in binding. We recommend crosslinking after

printing per the protocols detailed below. The surface of the slide has a high affinity for binding so we strongly recommend you follow the cleaning and blocking protocols that follow.

2) General precautions

a) The protocols contained in this document are meant to be general guidelines only and some optimization may be required depending on the application, sample and equipment being used.
b) Refer to manufacturer supplied Material Safety and Data Sheets (MSDS) for proper handling and disposal of all chemicals.
c) We do not recommend exposing the slides to the following solvents and liquids:
   i) Aromatic solvents such as benzene, toluene, etc.
   ii) Chlorinated solvents such as dichloromethane, carbon tetrachloride, etc.
   iii) Hydrocarbon solvents such as n-Hexane, cyclohexane, etc.
   iv) Ethers such as diethylether, etc.
   v) Ketones such as cyclohexane, etc.
   vi) Vegetable and mineral oils and greases.
   vii) Prior to use test other materials and liquids containing long-chain alkyl groups in their structure.
d) 3dbiosurfaces® 3D NS-NC slide is for research use, not for in vitro diagnostic use without express written permission.
e) We do not recommend mixing the 3D NS-NC slide in solution with coated slides as the surface treatment of the coated slides may contaminate our slides.
f) When scanning plastic slides, including the 3D NS-NC slide, it is important to ensure that scanners that use mechanical force to secure the microarray slide in place while being scanned are properly calibrated to manufacturer's standards. Out of specification holders can distort the slide by moving the slide surface beyond the image depth window.

3) Storage and handling

a) Store the packaged slides at room temperature and use prior to the expiration date.
b) Open and use the slides in a clean environment to avoid particle build-up on the printing surface.
c) Avoid direct contact with the printing surface to minimize contamination and abrasion of the coated surface.
d) Once the package is opened, slides should be stored under inert condition and protected from light at room temperature.
e) Slides should be kept in the slide box with the cover closed until used to prevent particle build-up on the printing surface.

f) We do not recommend washing the slides prior to use as liquids may be retained in the nanostructures.

4) Reagents required

PRINT SOLUTION Phosphate Buffered Saline (PBS) solution pH 7.4 with a final protein concentration of 0.05 – 0.2 mg/ml An alternative is 1 X PBS 7.4 PH+2.5% glycerol + 0.0006% Triton-X 100
BLOCKING WASH SOLUTION A 1% (w/v) solution of Pluronics F127 + 0.5% (v/v) in distilled water. As an alternative, purchase a 500ml bottle of the Blocking Wash Solution. It is available on the Product page
BLOCKING SOLUTION It is not necessary to use an additional blocking solution. The Blocking Wash Solution is sufficient to prevent non-specific binding.
BINDING SOLUTION PBST (PBS plus 1% Tween), 1:500 dilution of primary antibody and 1:250 dilution of fluorescent tagged antibody in PBS plus 1% Tween 20

5) Equipment required

a) UV Crosslinker (254 nm)
b) Humidified hybridization chamber.
c) Centrifuge with slide holders or compressed nitrogen gas for drying slides.
d) Slide dish and rack combo for washing slides.

6) General information regarding printing

a) 3dbiosurfaces® 3D NS-NC slide is compatible with all microarray printing or spotting methods, including contact printing and piezo technologies. Set up the arrayer according to the manufacturer's recommendations. You may have to re-calibrate the distance

between the slide surface and the spotting pins if you are using contact printing.

b) We recommend a vacuum hold down of the slide to the arrayer.

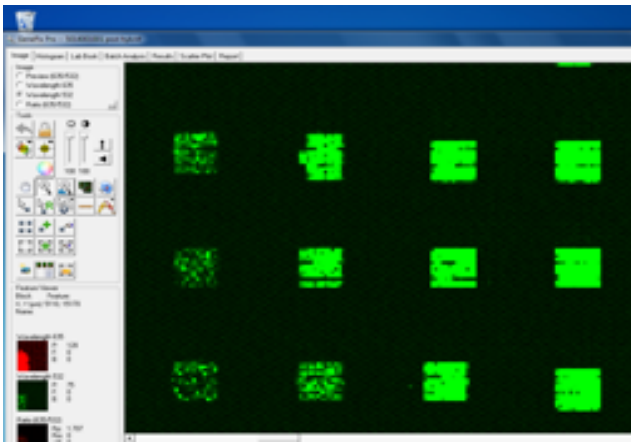
7) Steps prior to printing

a) Prepare the reagents.
b) Transfer the print solution and transfer to a multiwell plate in preparation for printing.
c) Dose the slides in a UV crosslinker immediately before printing at 1500 microjoules/cm^2 2 times.

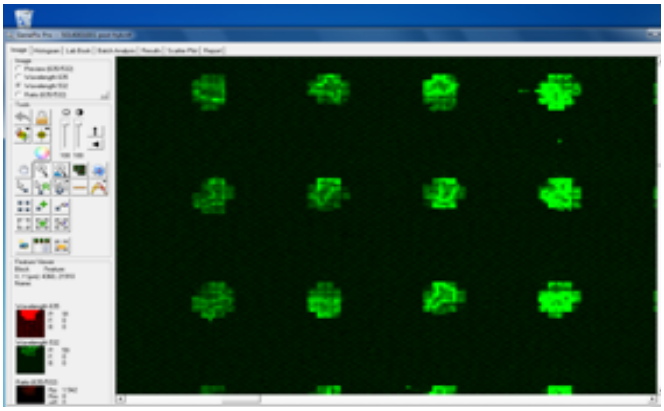
8) Printing

a) Print the slides at 50% relative humidity at 20 to 25 degrees C.
b) It is important that slides fully access the nanostructured surface of the slide for optimal performance. The spots should appear square with no gaps within the spot or coffee ring effect when viewed in a fluorescent scanner as shown below. If the spots do not meet this standard, then the print buffer can be adjusted or the number of times UV cross-linking dosing is performed adjusted to make the surface more wettable. This will also improve binding to the surface of the slide.

Good Spot Morphology



Poor Spot Morphology



9) Protein immobilization

a) Incubate printed slides in humidity chamber at room temperature for 1 hour.
b) If not proceeding to binding immediately, Incubate the slides at ambient temperature (40 to 50% humidity at 20 to 25 degrees C) overnight.

10) Crosslinking slides

a) Crosslink the slides in a UV crosslinker at 1500 microjoules/cm^2 2 times. You can experiment by changing the number of dosing is repeated to optimize this process but too many times may damage the proteins.
b) Dry the slide by centrifugation (1000 rpm for 2 minutes) or in an oil free air or nitrogen stream to avoid water stains on the slide surface. (You may wish to scan the slide for quality control purposes.)

11) Blocking/Washing

a) Wash slides using the **3dbiosurfaces Blocking Wash Solution** to remove unbound probe molecules and buffer substances. This solution is designed to also block any re-attachment by unbound molecules.
   i) Rinse (with agitation) for 5 minutes in the **3dbiosurfaces Blocking Wash Solution** at room temperature. Decant the solution. Repeat 1 time with **3dbiosurfaces Blocking Wash Solution**.
   ii) Rinse (with agitation) once for 5 minutes in diH2O at room temperature. Decant the solution.
b) Proceed to the Binding step

12) Binding

a) Pipette the appropriate volume of binding solution into a hybridization station or slide box, or onto the surface of the blocked slide under a cover slide for 2 hours or your own binding protocol.

13) Post-binding washing

a) Place the slide into a slide rack and immerse into a dish containing the Post Binding Wash Solution (usually 0.2X PBS). Wash in the wash solution for 1 minute at room temperature. Decant the solution. Repeat.
b) Wash (with agitation) for 10 minutes in diH2O at room temperature. Decant the solution. Repeat once.
c) Dry the slide by centrifugation (1000 rpm for 2 minutes) or in an oil free air or nitrogen stream to avoid water stains on the slide surface.
d) Protect the slide from light, dust and abrasion of the array surface until ready for scanning.

14) Important information about patents

The purchase of 3dbiosurfaces® products does not convey any license of any other company's patents. For all applications 3dbiosurfaces® makes 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.