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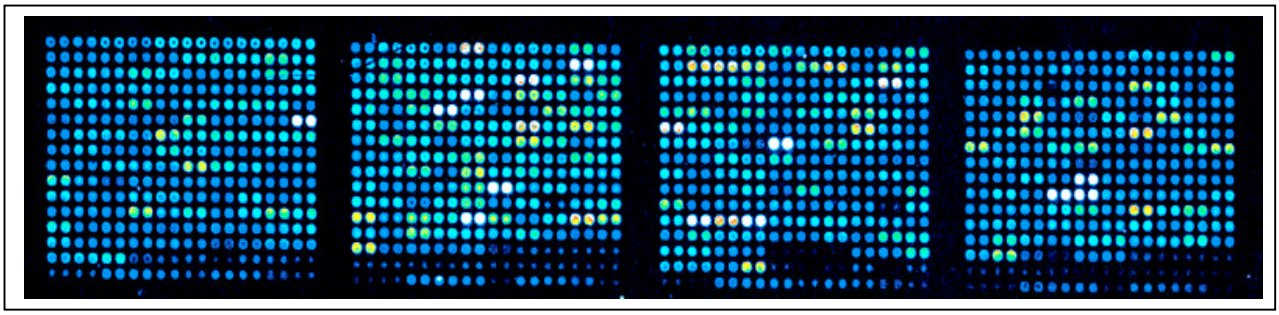

PROTEIN PROTOCOL

SuperChip™ Epoxy Silane
C50-5588-M20

Slides / Specialty Glass | 20 Post Road | Portsmouth, NH 03801-5691 | (800) 258-0834 (603) 431-8410 (603) 427-0881 Fax | www.thermo.com/microarray

Introduction:

Instructions for performing protein microarrays on Epoxy silane substrates

Thermo Scientific Epoxysilane microarray substrates are manufactured using premium quality glass drawn for it's superior flatness and low fluorescence properties. Manufacturing process are designed and controlled to ensure pristine glass surfaces and extremely tight tolerances. The ultra-flat surface provides an unmatched tolerance of <10 um across the diagonal of the slide as well as industry-leading topography. See Addendum for details.

The Epoxy surface modification interacts with amino acid side chains to form covalent bonds. Substrates are coated by proprietary processes in a highly controlled cleanroom environment. This ensures that the Epoxy groups form a monolayer over the entire surface of the slide. This attention to detail provides the highest probability for uniform and consistent microarrays. Substrates are available with and without barcoding. Bar codes are silk-screened onto the slides for a permanent, non-contaminating archiving tool.

Storage and Handling:

1. Store unopened packs at room temperature (20-25^{0}C) and use within recommended time frame (1 year from date of manufacture).
2. Open foil barrier bag in clean environment, using care to avoid dust, and particles near the array surface.
3. Upon opening, substrates should be stored in a dessicated container, protected from light.

Materials Needed:

1. Print Buffer: PBS @ pH 7.0-8.0
2. Blocking Buffer: PBS +1% BSA +/- 5% Sucrose, or 1% BSA in PBST, or 3% nonfat milk in PBST
3. Wash Buffer: PBS + 0.05% Tween 20
4. Rinse: 1:10 PBS

Equipment Required:

1. Humidified hybe chamber or 'tupperware' container with a layer of NaCl soaked towelettes; tight sealing cover to provide nominal humidity of about 75%
2. Centrifuge with slide holders, or compressed nitrogen…for drying slides
3. LifterSlips™ sized to cover entire array with extra space at all sides
4. Coplin jars, slide dish, or racks for washing slides

Printing Arrays:

Dilute antibodies (or proteins) to a final concentration of 50-100 µg/ml in the print buffer. Thermo Scientific SuperChip microarray substrates are compatible with all microarray spotting technologies that require 25x75x1mm slides. In addition, custom sizes and coatings are available upon request.

Protein Immobilization:

Print proteins at moderate relative humidity, 50-60%, and place printed substrates in a humidity chamber at room temperature for 1-2 hours.

Blocking:

Block slides in required blocking solution for 30-60 minutes at room temperature with rocking or slow shaking, if possible.

Assay and incubation protocol:

For incubation of proteins, make up target solutions in either PBST or blocking buffer, if there is concern about non-specific binding.

1. Dilute ***labelled*** target sample in appropriate volumes to cover the entire printed array.
2. Place LifterSlips over the slides; using pipette, slowly release labelled target under short edge of LifterSlip and let the chamber fill by capillary action, being careful to avoid bubbles.
3. Incubate slides for ~1 hour at room temperature.
4. Remove the LifterSlip and place slides in slide rack or Coplin jar. Fill dish or jar with PBST, enough to immerse the arrays completely.
5. Wash, with shaking, for 10-15 minutes.
6. Repeat.
7. Wash in 1:10 PBS for 10-15 minutes, with agitation, or shaking.
8. Dry slides using compressed nitrogen, oil-free air, or a centrifuge to avoid water marks.
9. Scan the arrays right away, or store protected from light, in a clean, dust-free area, until ready for scanning.

IMPORTANT PATENT INFORMATION

Thermo Scientific's SuperChip™ microarray substrates and accessories may be used in connection with the manufacture, use and/or analysis of oligonucleotide arrays under patents owned by Oxford Gene Technology Limited or related companies ("OGT"). Thermo Fisher Scientific does not have the right to pass on a license under any such patents. The user should check with OGT to determine whether or not it is necessary to obtain a license for the manufacture, use or analysis of oligonucleotide arrays, and if so, secure one.
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