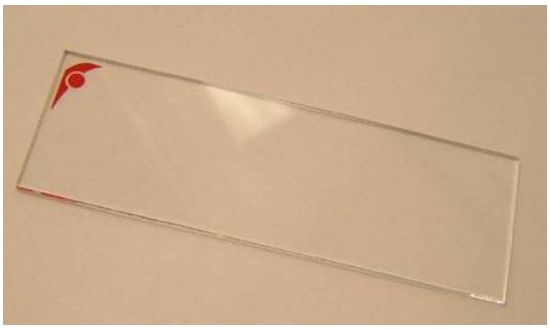
Fluorous Modified Glass Slides, 25x75x1.0 mm



Available in either plain or barcoded varieties these slides are made, packaged, and shipped to strict standards suitable for microarray formation. These slides provide the perfect media by which to immobilize your fluorous tagged substrates for microarray analysis. Fluorous microarrays offer very low background fluorescence, exceptionally low non-specific binding, simpler workflows, and excellent spot morphology. Available in boxes of 10 slides each, these slides are standard 25.10 x 75.36 x 1.00 mm.

Product Description: Fluorous Modified Glass Slides, 25x75x1.0 mm
FTI catalog #: 850-9100

Protocol for Small Molecule Immobilization:

From Vegas, A. J.;*et al*, *Angew. Chem., Int. Ed.* 2007, 46, (42), 7960-7964.

Microarray Preparation: Fluorous tagged small molecules were arrayed onto fluorous-functionalized glass slides (Fluorous Technologies, Inc,) using an OmniGrid®100 Microarrayer (Genomic Solutions) outfitted with an ArrayIt™ Stealth 48-pinprinthead and SMP3 spotting pins (TeleChem International, Inc.) as described previously (Bradner et al, Nature Protocols, 2006, 1, 2344). The microarrays contain 360 printed features with 10 replicate subarrays of 6x6 features with 300-µm center-to-center spacing. Array features typically displayed diameters of 80 to 150 µm. Solutions of small molecules (~ 2.5-10 mM in DMSO) were printed from 384-well polypropylene plates (Abgene). Note that proper pin washing protocols are critical for fluorous array manufacturing. Failure to clean away hydrophobic compounds, especially dyes, results in carry-over of the samples. Prior to printing, the pins were sonicated in dimethylformamide for 30 minutes. Within the print run, each pin was washed 5 times (incubated for 6 seconds) in dimethylformamide, and vacuum dried between picking up samples from the wells to minimize carryover. 50-100 arrays were printed in a given print run. Quality control for each print run involved scanning arrays prior to screening and looking for the presence or absence of various fluorescent control features as well as screens to detect a known protein-ligand interaction using conditions reported previously (Bradner et al, *Chem Biol,* **2006**, *13*, 493).

Small molecule microarray Screens with Pure Proteins. Printed slides were incubated with purified HDAC2, HDAC3/NCoR2, and HDAC8 (BPS Biosciences) at a protein concentration of 1 µg/ml in HDAC assay buffer (50 mM HEPES pH 7.4, 100 mM KCl, 0.005% Tween 20) in a 4 well dish (Nunc). Each slide required approximately 7 mL of protein solution to fully cover the surface. Slides were then gently agitated on a VWR Rocker (speed value 15, tilt value 6) at room temperature for 30 minutes. The protein solution was then discarded and slides were immediately incubated with Alexa-647 conjugated anti9 pentaHis antibody (Qiagen) diluted 1:1000 in HDAC assay buffer (7 mL per slide). After agitating slides with antibody at room temperature for 30 minutes, the antibody solution was discarded and slides were quickly rinsed (< 10 seconds) with HDAC assay buffer once followed by a rinse of distilled water. Slides were then dried by centrifugation for 15 seconds. Slides were then immediately scanned on an Axon 4000B or Axon 4200A scanner at 635 nm to detect binding of the Alexa 647-labeled antibodies. To analyze the array features, total fluorescence intensity values were calculated for a set 160-µm diameter centered over each feature using GenePix Pro 6.0 software (Axon

Instruments, Inc.).

2. Direct spotting of the wash or eluent to a MALDI plate can be conducted. Prewet the target with a amount a small amount water to prevent smearing on the plate. Alternatively collect your wash or eluent in a clean tube.

Protocol for Carbohydrate Immobilization:

From Ko, K.-S.; Jaipuri, F. A.; Pohl, N. L., *J.Amer. Chem. Soc.* **2005,** 127, (38), 13162-13163.

Microarray Preparation: Fluorous-tagged carbohydrate compounds were dissolved in 60% methanol in water and spotted on the fluorinated glass slide using a robotic spotter (Cartesian PixSys 5500 Arrayer, Cartesian Technologies, Inc., Irvine, CA) at 30% humidity. The glass slide was dried in a humidifying chamber at 30% humidity for 2 h. Note: After drying, a slide was washed with HEPES buffer (3x) and then distilled water (3x) before drying and detection with ConA as described below. No visible difference was detected in fluorescence between the prewashed and unwashed slides, therefore the extra washing steps were eliminated and slides were used as printed directly.

Detection of protein-carbohydrate binding*:.* For Con A, FITC-labeled Con A in HEPES buffer (pH = 7.5, 10 mM), 1 mM CaCl2, 1 mM MnCl2, 100 mM NaCl, 1% BSA (w/v) or without BSA, and for ECA, FITC-labeled ECA in PBS buffer (pH = 6.8), 1.0% TWEEN 20, ECA (25 µg mL-1; EY Laboratories, Inc., San Mateo, CA) were used the detection of protein-carbohydrate interactions. For protein incubation, 0.5 mL of protein solution was applied to the printed glass slide. The arrays was incubated by using a PC500 CoverWell incubation chamber (Grace Biolabs, Bend, OR) and gently shaken every 5 min for 30 min. The slides were then washed three times with the incubation buffer followed by three washes with distilled water. The slides were subsequently dried for 30 min in a dark humidity chamber. The glass slide was scanned using a General Scanning ScanArray 5000 set at 488 nm.