HiPep Laboratories

Technical Note: Substrate Derivatization and Manual Arraying

How to Construct Own Microarrays Realizing Higher Throughput Research Accelerate Creativity from Exploratory Research to Applied Development

1. Overview

Biochips provide a remarkably broad range of applications through various combinations of capture molecules and samples (Fig. 1). In particular, the PepTenChip® substrate, made from amorphous carbon, offers properties ideal for biochip use, including high chemical resistance, low self fluorescence, low protein adsorption, and electrical conductivity. These features enable flexible design according to specific research objectives. (PAT & PAT.P) This report is an experimental protocols for immobilization of small molecules containing a carboxyl group (biotin) as capture molecules onto a carbon substrate via a linker. **A demonstration video is also available online for a reference.**

Manual Arraying -YouTube

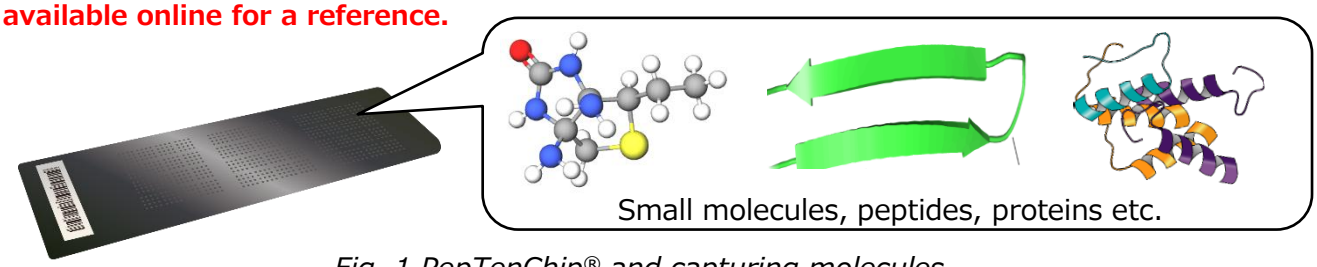

Fig. 1 PepTenChip® and capturing molecules

2. Derivatization

The carbon substrate surface is derivatized to match the capture molecule. Here, the carboxyl group substrate is used as the starting material and derivatized via a linker to the amino group terminus (Fig. 2).

2-1 NHS Derivatization

1. Dissolve 34.5 mg of *N*-Hydroxysuccinimide (NHS) in 10 mL of *N,N*-dimethylformamide (DMF).
2. Place the carboxyl-terminated substrate into the reaction container (RV) (Fig. 3), add the above solution, and dilute to 30 mL with DMF (final concentration 10 mM).
3. Add 46 µL of *N,N'*-Diisopropylcarbodiimide and shake for 1 hour (final concentration 10 mM).
4. Discard the reaction solution and wash the substrate (NHS-terminated substrate) five times with DMF.

2-2 Amination (hexamethylene linker)

1. Place the NHS-substrate into the RV and add 30 mL DMF.
2. Add 42 µL hexamethylene diamine and shake for 1 hour. (Final concentration 10 mM)
3. Discard the reaction solution and wash the substrate 5 times with DMF and 5 times with methanol.
4. Dry under vacuum overnight in the dark.

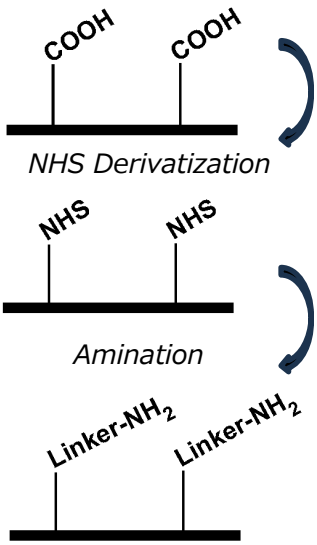

Fig. 2 Derivatization

TIPS

- We offer products with various pre-derivatized groups such as amino groups, maleimide groups, and bromoacetyl groups, tailored to experimental needs.
- Handle substrates while wearing laboratory gloves.
- We use Slide Fix™ slide jar (Evergreen Scientific) as RV.

Fig. 3 Substrate in reaction container (RV)

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3. Manual Arraying

The terminal functional group (-NH_2) of the derivatized substrate is conjugated with the capture molecule (D-Biotin) (Fig.4). The complement molecule, fixed via covalent bonding, becomes stable, enabling various washing operations.

1. Dissolve 2.4 mg D-Biotin and 3.8 mg HBTU in 1 mL DMF. (Final concentration of each: 10 mM)
2. Add 3.48 µL of *N,N*-Diisopropylethylamine to the above solution to activate it. (Final concentration 20 mM)
3. Dispense 0.1 µL of the reaction solution onto any desired location on the derivatized substrate (Fig. 5).
4. Place in a RV, shield from light, and let stand for 1 hour.
5. Rinse the substrate sequentially with DMF and H_2O.
6. Immerse the substrate in 50% isopropanol and sonicate for 15 min.
7. Dispense a small amount of 50% isopropanol onto the substrate and scrub clean using a PVA sponge or similar.
8. Rinse the substrate again sequentially with DMF and H_2O.
9. Dry the substrate by spin drying.

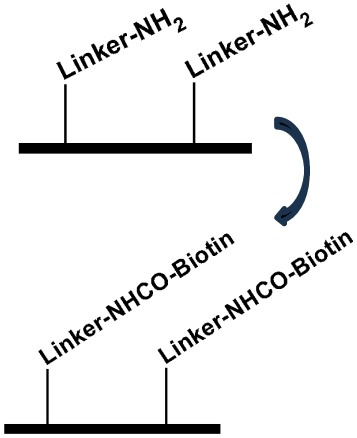

Fig. 4 Capture molecule immobilization

Proceed to assay operations →

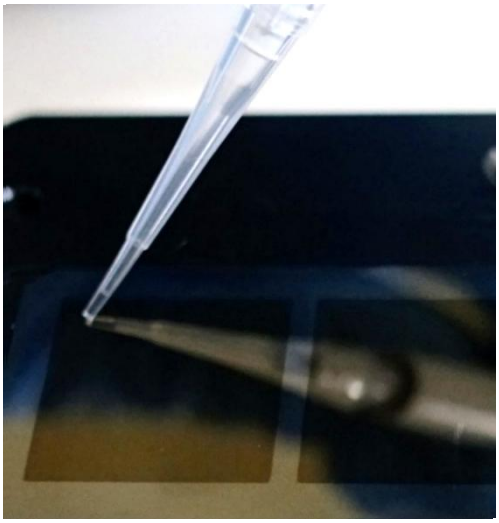

Fig. 5 Manual Arraying

TIPS
- It is recommend testing with the solvent alone beforehand.
- When preparing the reaction solution in organic solvents such as DMF, note that it may bleed slightly more than aqueous solutions due to surface tension and interactions with the coating.
- The optimal concentration and reaction time may vary depending on the capture molecule to be immobilized.
- We use AION PVA Wiping Materials (AION Co., Ltd.) as the cleaning sponge.

4. Specifications

Item	Specification
Material	Amorphous Carbon
Size	25.0 x 75.0 x 1.0 mm (Standard slide glass size)
Smoothness	<1 nm
Flatness	<10 µm
Amino Group Content	ca. 4-5 nmol/cm^2
Electrical Resistivity	40-45×10^{-6} Ωm

5. Products

P/N	品名
PTC-CA02-01	PepTenChip® CA (Carboxyl groups 3-Blocks)*
PTC-CA-01	PepTenChip® CA (Carboxyl groups fully derivatized)*

*Various functional groups, processing patterns, and substrate sizes are available.

References

1) Tominaga, Y., and Nokihara, K., *Anal. Methods*, 2025, **17**, 4590.
2) Nokihara, K., Chemical Engineering, 2024, **88**, 61-64.

Related Web sites

PepTenChip® https://hipep.com/?page_id=3662 https://hipep.com/?p=781
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