

NEW PRODUCT



PepTenChip® Guide Book

PepTenChip® a novel Bio-Chip focusing on protein detection, consisting of designed peptide and glycopeptide Libraries

Technical notes and Product Information

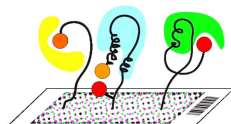
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PepTenChip® HOT LINE

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Representative of HiPep Laboratories



PepTenChip® a novel Bio-Chip focusing on protein detection, consisting of designed peptide and glycopeptide Libraries



Introduction

The major goal of biochips is their use for rapid and economical characterization of biological samples. We have devoted considerable effort to the development of novel bio-chip systems, which are urgently required in proteome research. We have proposed a novel biochip-concept for a protein detection system involving labeled structured peptides as capture molecules and the protein-fingerprinting method, which affords a barcode-like visualization, while the protein-protein interaction can be mimicked by a protein-peptide interaction [Reviewed in 1, 2, 3]. The novel chip, designated PepTenChip®, satisfies all these requirements for protein detection, specificity, reproducibility, sensitivity, easy handling, stability (storage/transport) and production economics. The novel chip material made from amorphous carbon has been developed, which has significant advantages over conventional glass slides. In practice our system has been shown to discriminate the structures of a number of different proteins. Important factors for peptide micro-arrays are the construction of peptide libraries, the use of chip materials with a suitable surface chemistry, the deposition of peptide solutions by an arrayer, detection and data mining. We have achieved several applications of the PepTenChip® [4, 5]. Although PepTenChip® and related products are getting into the market since 2010, optimization and further improvement are continuously carrying out.

Capture molecules

Arrayed molecules of PepTenChip® are designed peptides, which had been synthesized, purified and characterized one by one by the highly efficient manner in improved SPSS. These are several hundreds of labeled structured peptides of which secondary structures are confirmed by CD spectra. Hence the SPOT syntheses can not be employed, since the quality assurance is very important for quantitative analyses and reproducibility in biodetection. In fact one of the most important issues in bio-chips for protein detection is quantitative analysis.

Novel material for PepTenChip®

The novel chip material made from amorphous carbon has been developed [6], which has significant advantages over conventional glass slides, those are: (1) mechanically more stable, (2) chemically inert, (3) shows no self-fluorescence, (4) easy manufactured by laser drawing, thus micro-channel effects can be easily maximized (5) environmentally friendly (regeneration is easier) (6) higher thermal conductivity and higher electro-conductivity (heating & cooling can be easily performed, and can be used for electrochemical reactions). The grinding flatness is < 10 micron that has been performed by hard-disk technology. The surface chemistry which we have developed (patents pending) allows extremely low background and non-specific adsorption, uniform distribution of functional groups on the plate surface and their concentration on the surface is much higher than conventional materials, thus immobilization is much easier. Ion analysis using a detector of electric conductivity has been developed to confirm the amounts of functional group on the surface (patents applied for). This is indispensable for quality control of each bio-chip and is important as conventional X-ray photoelectron spectroscopy data do not correlate directly with reaction stoichiometry. The results revealed that the loading amounts on the present amino-carbon plate were ca 40 pmol/mm². Commercial slide glass has significant nonspecific absorption and not allowed to determine precisely. Together with present improved surface technology the arraying conditions have also been successfully optimized to give at least 10-20 amounts of immobilized peptides than previously.

To improve the spot formation tedious protocols have been proposed (metal ions or detergent was used as additives), since microarray spots often exhibit ring-like structures, which produce difficulties in quantitative characterization. The PepTenChip® gives uniformed spot-distribution using the improved surface technology. The basic chemical modification of our surface is through an amino group and further derivatization to carboxyl group, bromoacetyl group, succinimide ester, maleimide or biotin-streptavidin is easily achieved. Thus, the present material with special surface processing technologies provides excellent possible applications for bio-chips, sample trays for micro analyzers such as MALDI-TOF, ultra micro/nano-plates (assay), and micro reactors [7].

PepTenChip® Applications

The present paper describes the construction of structured peptide and glycopeptide libraries and the development of the novel chip-material using amorphous carbon with a special surface chemistry. Fluorescently labeled α -helical, β -loop and β -strand peptides (total number of ca 2500 and ca 100 O-glycosylated peptides) have been successfully prepared by improved solid-phase syntheses and characterization. The peptide solution (350 pico Liter), which contained Cys-residues for immobilization on the chip-surface, was deposited to form a ca 100 micron spot on a novel material described below by a Piezorrayer. Amorphous carbon plates were manufactured which are mechanically stable, chemically inert, none self-fluorescent, easily manufactured for laser drawing, and of high thermal and electro-conductivity. Our surface chemistry allows extremely low background and non-specific adsorption, uniform distribution of functional groups on the plate surface and a much higher concentration on the surface than conventional materials, thereby making immobilization much easier. The results revealed that the loading amounts on the present amino-carbon plate were ca 40 pmol/mm².

Bio-detection of several toxicant proteins has been achieved as an application of O-glycopeptides libraries. We demonstrate here that toxicant proteins show different responses to different glycopeptides using the present chip in a dose dependent manner and finger prints can be obtained against such toxicant proteins in solution using titer plates to give a similar detection range to that of conventional ELISA. In fact, solution assay requires larger amounts of arrayed peptides and analytes. We have constructed a bio-chip using the above plates. At the present time the optimized amounts of arrayed peptides on a chip was 9 femto mole and toxicant protein as an analyte was ca 20 ng. The assay has also been performed in the presence of 2% milk to simulate practical conditions. These results suggested that glycopeptide arrays show promising applications as a toxin detection tool.

1. Nokihara, K. et.al., *Kobunshi Ronbunshu*, 61, pp 523, **2004**. (in Japanese)
2. Nokihara, K. et.al., *Solid-Phase Synthesis & Combinatorial Chemical Libraries 2004*, Epton, R. ed.; Mayflower Scientific, UK, pp 83, **2004**.
3. Nokihara, K., *Future Materials*, 6, 42, **2006**, (Review in Japanese)
4. Nokihara, K et. al., *Peptide Science 2007*, Aimoto, S., Ono, S. eds.; Japanese Peptide Society, pp106, **2008**.
5. Kawasaki, T., Ohyama, T., Hirata, A. and Nokihara, K., *Bull. Chem. Soc. Jpn.*, 83, 799-801. **2010**.
6. Nokihara, K., et. al., *Peptide Science 2008*, ed. Nomizu, M., Japanese Peptide Society, pp95, **2009**.
7. Nokihara, K., et. al., *Peptide Science 2009*, Okamoto, K., ed.; Japanese Peptide Society, pp337, **2010**.

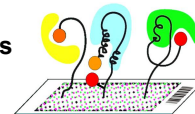


PepTenChip® *Designed peptide arrays & novel chip material*



Mission of Bio-chips: Rapidness • Energy saving • Higher efficiency in Bio-detection !

www.hipep.jp High throughput syntheses and characterization → High quality peptides



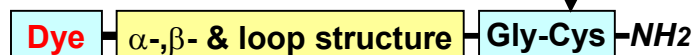
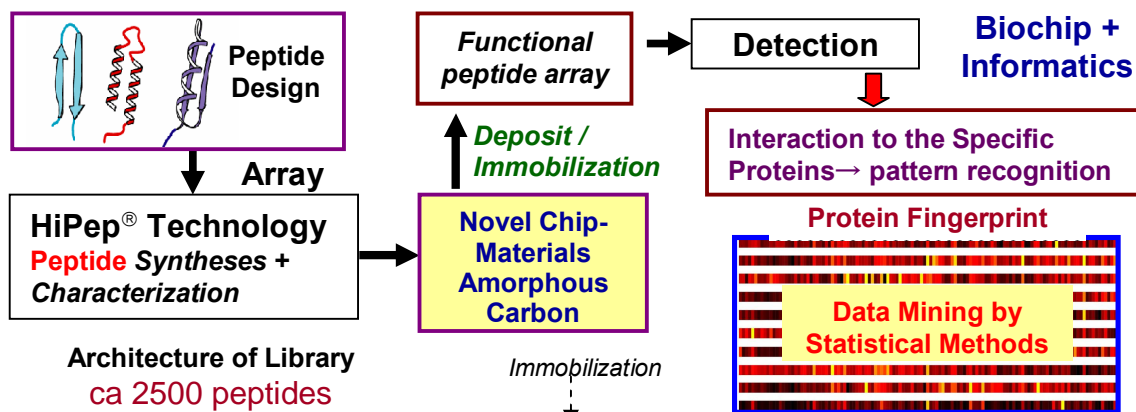
Bio-molecular Recognition

- Peptides are the core as transmitters via receptors & immunoresponses.
- Peptide-recognition = the mimic of protein-protein interaction.
- Proteins can be mimicked by peptides.
- Peptides can be designed and synthesized.
- Peptides can have structures.
- Large diversity is possible.
- Non proteinogenic amino acids can be used.
- Syntheses and characterization methods are established.

Practical Production Systems:

- ① Peptide design (Capture molecules)
- ② Peptide-Library (High quality peptides)
- ③ Chip materials
- ④ Immobilization (arrayer & surface chemistry)
- ⑤ Detection (Scanner, Microscope + CCD camera)
- ⑥ Data base (data mining)

PepTenChip® is an array-system of immobilized designed labeled peptides. PepTenChip® is Bio-chips for bio-molecule assay in the next generation, satisfies all these requirements for protein detection, specificity, reproducibility, sensitivity, easy handling, stability (storage/transport) and production economics.



Advantages of the novel amorphous carbon chip-plate

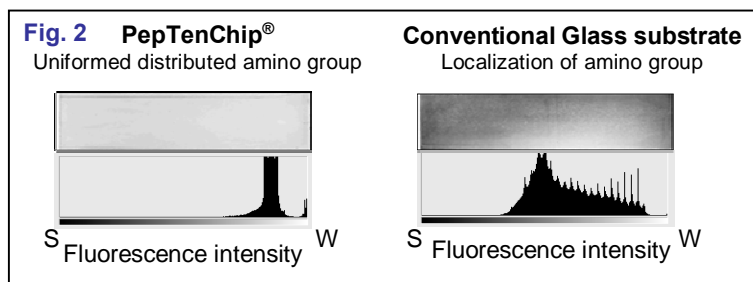
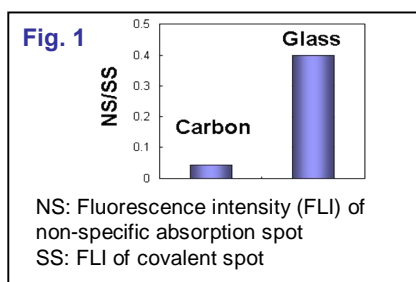
- ① Mechanically stable
- ② Chemically inert
- ③ Environmentally friendly (easier regeneration)
- ④ Easily manufactured by laser drawing;
Flat: Slide glass size, custom-made size
Ultra nano-well (Tailor made) eg 1~2 nano Liter
- ⑤ No self-fluorescence
- ⑥ Higher electro-conductivity
- ⑦ Lowest non-specific adsorption
- ⑧ Higher thermal conductivity (heat & cool)
- ⑨ Uniformed distribution of functional groups
- ⑩ Extremely low back ground
- ⑪ Original surface chemistry: different chemistry for derivatization and functional groups

Use for

- Bio Chip PepTenChip®; Assay Plates
- Micro-reactors
- Sample tray for microanalyzers
- Other industrial products

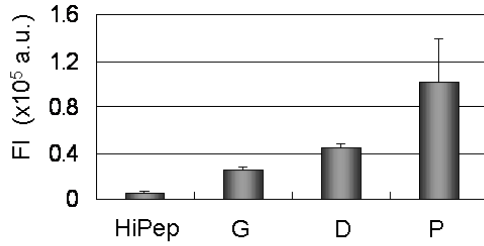
Advantages of PepTenChip® as bio chip

- ① Extremely low non-specific protein absorption than that of glass by the special surface technology (Fig. 1).
- ② Uniformed functional group distribution (Fig. 2).
- ③ Uniformed spot-formation on the microchips (Fig. 3).
- ④ Not easy to crack compared with the glass → Easy handling.
- ⑤ Functional group-rich on surface → Easy immobilization. Developed novel method for quantization the functional group amount: **Quality control (PAT.P)**





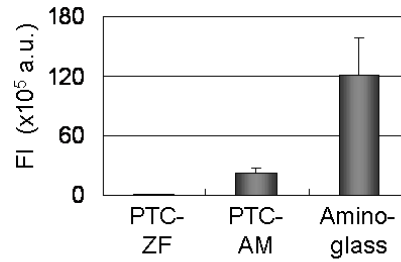
Comparison of self-fluorescence



HiPep: PepTenChip®
 G: Glass-slide
 D: DLC-Glass
 P: Plastic

Data obtained
 by a Fluorescence
 Laser Scanner

Non-specific Absorption of Fluorescent labeled BSA



PepTenChip® (PTC)
 made from Amorphous carbon
 PTC-ZF: without derivatization
 PTC-AM: aminated surface



PepTenChip®
 allows uniform distributed spots
 without any detergent and/or metal ion.

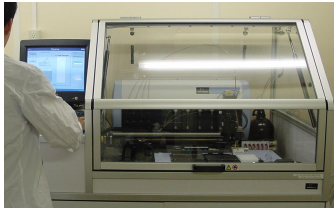
Fig. 3



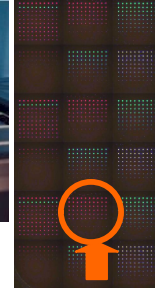
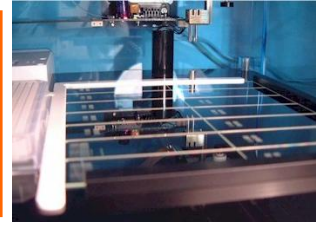
Glass substrate

Ring-like distribution of nano-drops gives difficulty in quantification. Ref. 8. X. Y. Zhu et al. JACS (2006) 128, 2768.

Immobilization using arrayer :
 Micro-Spotting pin or Piezorray
 Eg: Diameter 100 μm Φ
 350 pL , 10 μM (PBS)



We have manufactured
 Manual array-chip plates has
 been also manufactured

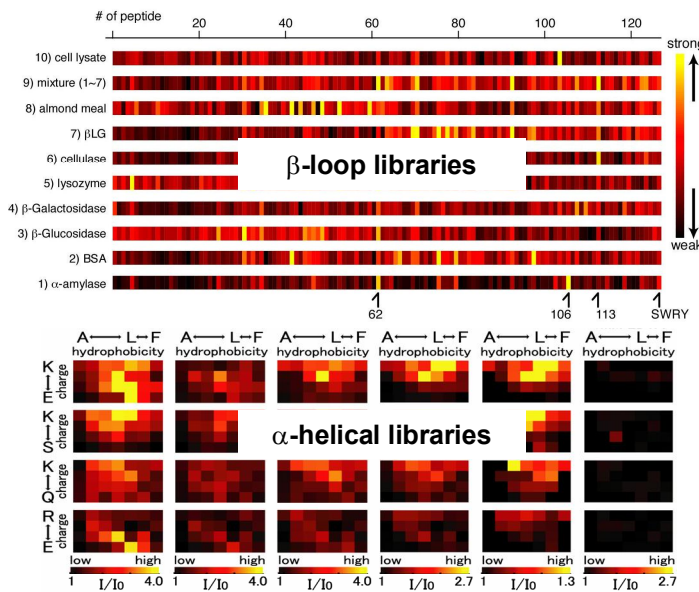


Ex 543 nm
 Filter:
 570 nm

4 X 4 mm

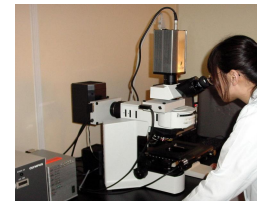
Detection

Pattern Recognition : Protein Fingerprint Method



Modified Commercial
 Scanner (for DNA-chip)

Fluorescent Microscopy
 + CCD camera



Novel detector: Two-Patents



Novel detector can also be used in BSL3, and 4 (almost maintenance free)
Concept: Easy handling, Portable, Low cost
Looking for collaborator/ manufacture under licencing.



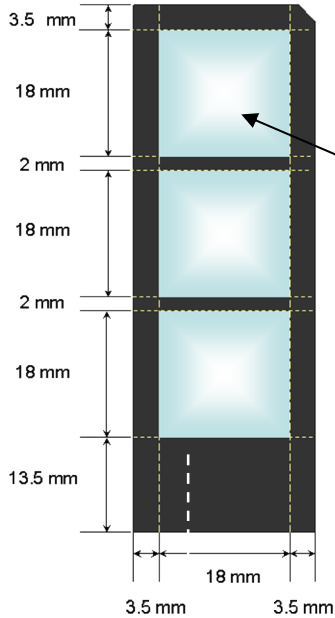
PepTenChip® Derivatization Pattern



Pattern A

Surface fully derivatized
P/N: PTC-AM-01-01

Array-chip plates of which surface are not fully derivatized. (Pattern B and C)



Pattern B

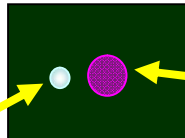
3 blocks derivatized
P/N: PTC-AM-02-01

Cover Glass Size

Pattern C

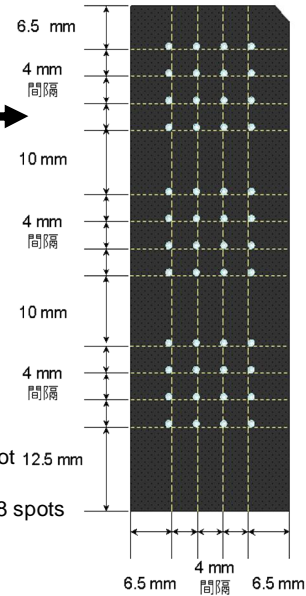
For manual array
48 spots derivatized
P/N: PTC-AM-03-01
aminated surface 1 mmΦ
spot generated by
a solution of the capture
molecule < 2 mm,

Only
1 mmΦ
aminated



Applied spot 12.5 mm
< 2 mmΦ
12 X 4 = 48 spots

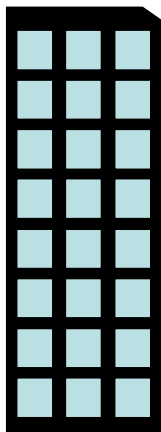
The derivatized area is smaller
than the spot diameter of 1 μL



⇒ Manual spot size can be prepared uniform

Tools for easier recognition/incubation

Much smaller amounts of analytes can be applied when the following items are used



Pattern D

24 blocks derivatized:
P/N: PTC-AM-04-01



Incubation Cassette
For 24-Block Plate
P/N IC24-01
Each well 50 μL



Gaskets set
P/N ICG24-01

0~70°C
used

Ex: 48 spots
manual array



Small amounts
of water against
dehydration

Slide Chamber ex: Pattern C (P/N: PTC-AM-03-01)



Color= B, P, N, G, Y, R

P/N ICT(color=B~R) 01-01



Bio-detection and Microarray services

Analysis of array with fluorescent labeled peptides requires fluorescent microscope/scanner.

We provide Analytical Service of Bio-detection (details separate pages).

Analysis of captured molecular by MALDI-TOF-MS: recognized-molecular on array chip can be analyzed by MALDI-TOF-MS (ref. below)

Manufacture of tailor-made array eg customer's library is possible.

Ref. Nokihara, K., Hirata, A., Ohshima, T., Sogon, T., Kawasaki, T., Takebayashi, Y., Oka, Y.

Peptide Science 2009, Okamoto, K., ed.; Japanese Peptide Society, pp 337-340, 2010.



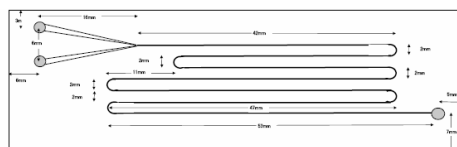
Microreactors (application of an amorphous carbon plate)

Carbon has higher electro-conductivity (electrochemical detection), higher thermal conductivity, higher chemical stability, easier manufacturing than glass.

Micro-channel

Large specific interfacial area.
 Large force to fluid=Control
 Mass transfer to surface=
 Reaction, detection •short
 molecular diffusion distance
 •Large specific interfacial area
 (solid-liquid, liquid-liquid
 interface)
 •Small heat capacity

Example Micro-channel
 25 X 75 X 1.0 mm



Combi-chem → small scale multiple
 Multiple parallel → large scale

Benefits ★ Resource saving, Cost saving
 ★ Micro scale syntheses
 ★ Micro size effects

Micro-fluid device
 Effect of scale down
 Effect of micro scale

**Collaborations
 are welcome**

Novel material amorphous carbon plates

Specific gravity 1.4892 g/cm³ Standard chip size 25 x 75 mm (tolerance ±0.1 mm)
 thickness 1 mm (±0.025 mm) Upper left corner (1.5 x 2.0 mm)

PepTenChip® Quality Assurance

PepTenChip® AM surface; Stable at least 3 months under the protection from light.

○: Stable	Protected against light, 25 °C	Not protected against light	
		Laboratory, 25 °C	Outside of the laboratory, 25-35 °C
1 week	○	○	○ (tested in the summer time)
1 month	○	○	×
3 months	○	○	×

PepTenChip® AM with fluorescent labeled (TAMRA) arrayed: Poor stable without protection against light. Under protection of light, it is stable at least 3 months (25 °C)

- Storage of PepTenChip® AM (Amino group, Carboxyl group etc. surface derivatized)
 1. Vacuum-packed with shading film used for shipping: Stable at least 3 months from the production date
 2. It is recommended that storage below room temperature and used immediately after open the seal for packing.
- Storage of PepTenChip® arrayed with fluorescent labeled peptides
 1. Vacuum-packed with shading film used for shipping: Stable at least 3 months from the production date
 2. It is recommended that stored in freezer (below -20 degree).
 3. Before use acclimatization at room temperature and immediately used when package is opened



PepTenChip® Product Line



The list price is per plate. Individual quotation is given for tailor made chips.
Prices are subject to change without prior notice. Status 2010 June 30

Product name	Surface on chip	P / N	Description and possible Applications	List Price (Yen)
ZeroFlat	Without derivatization	PTC-ZF-NN-01	Basic use :Free processing by users (Easy handling). Possible to make wells or micro-channels. Special application : Substrate for photovoltaic generation (fuel battery separator), components of semiconductor (Carbon wafer), aerospace materials.	25,000
ZeroWell	Well, without derivatization	PTC-ZW-NN-01		65,000
AM	Amino group (G)	PTC-AM-NN-01	The surface covered with amino groups. The very low non-specific adsorption. Basic use : Immobilization with anionic polymers (eg DNA etc.) Modification to the carboxyl group.	40,000
CA	Carboxyl G	PTC-CA-NN-01	Basic use : Immobilization with cationic polymers by the ionic bond. The amino group can be immobilized thru amide bonds.	45,000
MI	Maleimide G	PTC-MI-NN-01	The maleimide group is useful for sulfhydryl reaction (eg Cys).	60,000
BR	Bromoacetyl G	PTC-BA-NN-01	Immobilization through sulfhydryl group.	45,000
SA	Streptavidin	PTC-SA-NN-01	Immobilization for biotin-labeled biomolecules.	70,000

PepTenChip®PA ~ Bio-chips in the next generation ~

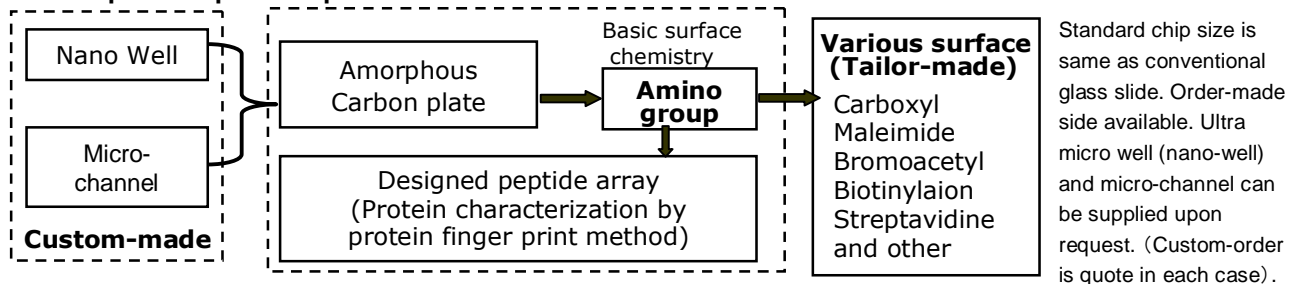
Peptide array designed peptides are arrayed as capture molecules by covalent bond for detection.

The protein can be identified through constructing the data base by the protein fingerprint method. We can also custom-made Chip-plates Upon requests. The standard size of the chip-plate is the same as conventional slide glass. Any size can be made.

Product name	P / N	Description and Application	List Price per plate (Yen)
PepTenChip®PAH	PTC-PAH-01/02-01	About 500 kinds of labeled a-helical peptides	250,000
PepTenChip®PAL	PTC-PAL-01/02-01	About 500 kinds of labeled b-loop peptides	250,000
PepTenChip®PAS	PTC-PAS-01/02-01	About 400 kinds of labeled b-sheet peptides	250,000
PepTenChip®PAG	PTC-PAG-01/02-01	About 100 kinds of labeled glycopeptide	250,000
PepTenChip®PAX	PTC-PAX-01/02-01	Designed peptide library for customer	Please inquire

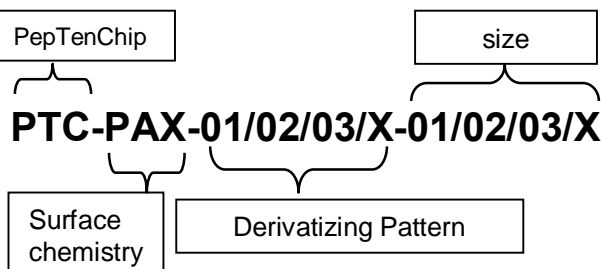
- The PepTenChip® plates are suitable also for array consisting of DNA, sugar, antibody, antigen and low molecular compounds.
- For collaboration PepTenChip® will be supplied free. (ref. alliance) Trial can be delivered also free under MTA.

PepTenChip® Lineup



P/N Chart

01	Fully derivatized
02	3 Block derivatized
03	Manual array
X	Custom-made



size	size
01	25 x 75 mm
02	20 x 30 mm
03	10 x 10 mm
X	Custom-made



PepTenChip® contract analysis



The price indicated in the table is Japanese yen, excluding tax. We charge the analyses without desired results.

The chip-plate used in the contract analyses is excluded. Consultation fee is calculated separately.

Besides this, collaboration research or system developments are separately calculated. Multiple analyses will be quoted the reduced price.

The analyses for clients who purchases PepTenChip® PA (designed peptide array) are 50% reduction during campaign.

Content	Details	Initial price (JPY)
Arraying	Arraying customer's own molecules on to PepTenChip®	Upon request, price is dependent on number of spots
Detection by fluorescent microscopy	Clients own-chip analyzed by fluorescent microscopy and CCD camera. Characterization of standard software, PepTenChip® analysis system	
Detection by fluorescent scanner	Clients chip fluorescent analysis by laser-scanner then analyze by the device attachment standard software Device: Remodeled CRBIO Ile (Hitachi Software Engineering Co., Ltd.)	
MALDI-TOF	MALDI-TOF analysis of chips from clients own made MS/MS analysis solely indicated in the separate price list Device: UltraFlex III TOF/TOF (Bruker-Daltonics)	
Data interpretation	Interpretation data from above analysis (No longer than 3 hours)	50,000~

Array conditions Tailor-made available : call us for details

Sample	From clients	Number of sample	Under consultation
Spot diameter	< 100 μm	Distance between spots	200 ~ 1500 μm
Number of n	n=3 to 10	Number per order	1 to 25
Device	SpotBot™ (TeleChem)	Material	PepTenChip®
	Piezorray™ (PerkinElmer)		
Material	PepTenChip® or from clients	Delivery	2 to 3 weeks
Others: followed our standard contract format			

★ **Tailor-made available**: call us for details, R&D, collaboration research

PepTenChip@hipep.jp or info@hipep.jp TEL +81-75-813-2101 skype: hipepkyopto

PepTenChip® Team: Dr. Ohyama, Dr. Hirata, Dr. Nokihara

Key point

- ◎ In the future, demand of molecular diagnosis using bio-chip will be dramatically increased.
- ◎ Bio-chip promises rapid, energy saving, highly efficient analyses also ON-SITE.
- ◎ Designed peptide array is a sensor device.
- ◎ PepTenChip® is Bio-chips for characterization for proteins in the next generation, and satisfies all requirements for Bio-detection; Specificity, Reproducibility, Sensitivity, Easy Handling, Stability (storage/transport), Production Economics.

PepTenChip® Applications

- ① Research in proteome, clinical medicine, environmental analyses.
- ② Quick diagnosis contributes in prognosis, early detection and prevention of disease; home medical care, on-site examination.
- ③ Environmental monitoring
- ④ Safety control for food, agricultural chemicals.



Publications related to Peptide Array since 2001

Dr. Nokihara and his co-workers, HiPep Laboratories, as of December 2010



2001

Nokihara, K., *Chemistry and Biology*, Gakkai Shuppan Center Co. Ltd, Tokyo, **2001**, **39**, 56-62, Recent Progress in Combinatorial Chemistry: Combinatorial Analyses for Characterization of Libraries (Hyphenated Technology)

2002

Nokihara, K., and Mihara, H., *Protein, Nucleic Acid and Enzyme*, Kyoritu Shuppan, Co. Ltd., **2002**, **47**, 626-632, Protein Chip, an Innovative High Throughput Method for Detection of Functional Proteins (*Review in Japanese*)

Nokihara, K., *Shimadzu Review*, **2002**, **58**, 137-14, Physico-chemical Characterization and Purification of Combinatorial Chemical Libraries - focusing on high- throughput- (*Review in Japanese*)

Usui, M. Takahashi, A. Ueno, Nokihara, K.; H. Mihara, *Peptide Science 2001*, Aoyagi, H. ed.; The Japanese Peptide Society, **2002**; pp 405-406. Construction of a Protein-Detection System using Designed Peptides with Fluorescent Labels

Mihara, H.; Takahashi, M.; Usui, K.; Ojima, T.; Ueno, A.; Nokihara, K. *Peptides 2002*, Benedetti, E., Pedone, C. eds.; Edizioni Ziino, Napoli, Italy, **2002**; pp 564-565. Protein-detection systems using structure-based peptide libraries and peptide microarrays

Usui, K.; Takahashi, M.; Ueno, A.; Nokihara, K.; Mihara, H. *Peptides 2002*, Benedetti, E., Pedone, C. eds.; Edizioni Ziino, Napoli, Italy, **2002**; 646-647. Microarrays with α -helical peptides for protein detection using a FRET technique

2003

Takahashi, M.; Nokihara, K.; Mihara, H. *Chemistry and Biology*, **2003**, **10**, 53-60. Construction of a protein-detection system using a loop peptide library with a fluorescence label

Mihara, H.; Takahashi, M.; Usui, K.; Ojima, T.; Nokihara, K. *Peptide Science 2002*, Yamada, T. ed.; The Japanese Peptide Society, **2003**; pp 109-110. Structure-designed peptide microarrays for protein detection

Usui, K.; Takahashi, M.; Ojima, T.; Suzuki, M.; Nokihara, K.; Tamiya, E.; Mihara, H. *Peptide 2003 Revolution: Genomics & Therapeutics*, Chorev, M., Sawyer, T. K. eds.; American Peptide Society, **2003**; pp 258-259. Peptide microarrays using structure-based peptide libraries for protein chips

2004

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