GENE ON BIOTECH

Epitope Mapping
Experiment Analysis Report

User name: OOO OOO

Peptide array: OOO OOO

Sample name: OOO

2020. O. O.

1-1 Experiment Details

GENE ON BIOTECH

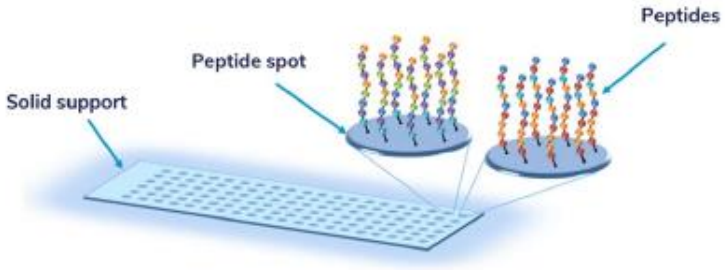
Experiment schedule	5/11: Sample receiving. 6/1: Sample checking 6/1-3: Pre-treatment 6/1-6/5: Background Test 6/8-12: 1st Peptide array experiment(0.5ug/ml) 6/15-19: 2nd Peptide array experiment(1ug/ml) 6/22-26: 3rd Peptide array experiment(1.5ug/ml) 6/29-7/2: Analysis and data mining 7/3: Sending experiment report with raw data
Purpose	Epitope mapping
Chip	OOO
Sample name	OOO Antibody
Reagents and devices used	Reagents: PBST pH. 7.2 (Bio Solution) Bovine Serum Albumin (IgG-Free, Protease-Free; Jackson ImmunoResearch Laboratories) Devices: InnoScan 710 Microarray scanner, Innopsys GenePix 4100A Microarray Scanner, Molecular Devices

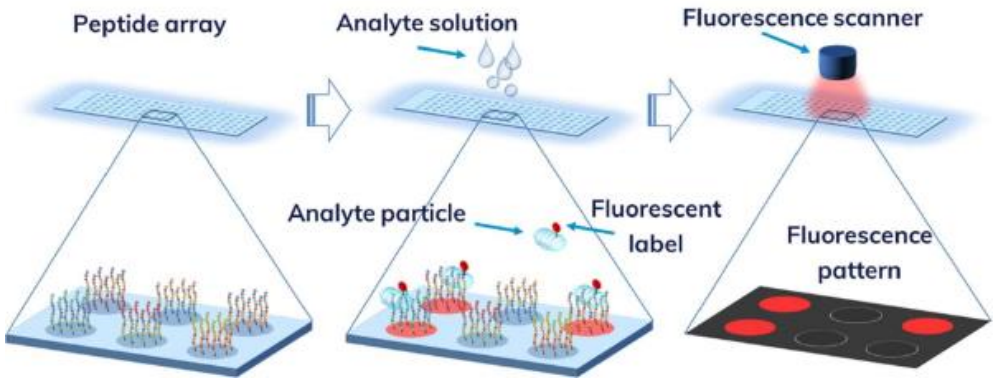
1-2 Introduction

GENE ON BIOTECH

Introduction

A peptide microarray is a collection of various amino acid sequences arranged on a solid support in a spot array format. Each spot of the microarray represents a multitude of identical peptides, whereas various spots generally contain different peptides. The amino acid sequence in each spot is defined during peptide microarray fabrication, while the information on their spatial allocation constitutes a molecular library of a given peptide microarray. Since the molecules are covalently bound the substrate, a parallel high-throughput screening of their chemical, biological, or functional activity becomes possible.





The peptide array workflow

A peptide array containing a known molecular library is incubated with a biological sample, which can be patient or animal sera, enzymes, antibodies, cells, or cell lysates. The analyte components interact with the peptides displayed on the substrate and bind to those molecules that demonstrate sufficient affinity. The non-bound analyte components are removed during subsequent washing steps. In order to visualize the spots containing the peptides with high affinity to the biological sample, the analyte can be fluorescently labelled prior to the incubation.

Alternatively, the analyte components bound to the peptides can be tagged with fluorescently labelled antibodies in an additional staining step. Both approaches enable visualization of the remaining analyte on the microarray by fluorescence readout techniques. By collating the resulting fluorescence spot pattern with the peptide microarray library, both binding and nonbinding amino acid sequences can be identified.

2-1 Experiment Protocol

GENE ON BIOTECH

A. Background test: Pre-staining with secondary antibody / Streptavidin and control antibody

The secondary antibody or streptavidin used for staining of your sample may interact with the peptides printed on the microarray. To discriminate such background interactions from sample specific signals, It is recommended pre-staining with secondary antibody / streptavidin and pre analysis Peptide Microarray. Pre staining should be performed only once per microarray design. Background signals can be subtracted from signals obtained from your sample during data analysis.

1. Place the microarray in the incubation chamber and/or assemble the incubation tray. Make sure you can properly read the microarray number at the bottom of the slide.
2. Incubate the peptide microarray for 15 minutes at room temperature in washing buffer using an orbital shaker at 140 rpm for optimal liquid surface coverage. Check carefully for incubation tray leakiness and tighten the thumbscrews if necessary. Aspirate the buffer.
3. Incubate the peptide microarray for 30 minutes at room temperature with blocking buffer. Use an orbital shaker at 140 rpm. Aspirate and remove the buffer when completed.
4. Stain with secondary antibody or streptavidin and control antibody diluted in staining buffer for 45 minutes at room temperature using an orbital shaker at 140 rpm, protected from light.
Dilute the secondary antibody or streptavidin and control antibody as described in “Recommended Sample and Secondary Antibody Dilutions”. Completely aspirate and remove the antibody solution.
5. Wash the peptide microarray 3x for one minute with washing buffer using an orbital shaker at 140 rpm, protected from light. Completely aspirate and remove the buffer.
6. Disassemble the incubation tray and carefully take out the microarray from the incubation chamber.
7. Directly dip the microarray 2 - 3 times into dipping buffer filled in a beaker. If necessary, slightly tilt the beaker. Repeat this step until all visible contaminations (e.g. PBS residues or dust) are removed.
8. Carefully dry the microarray completely in a pressurized air stream from top to bottom.
9. Scan the peptide microarray according to guidelines of your scanner using the appropriate fluorescence channels.

B. Incubation with the sample

10. Re-assemble the incubation tray as described earlier.
11. Equilibrate the microarray in the staining buffer for 15 minutes at room temperature on an orbital shaker at 140 rpm. Aspirate and remove the buffer when completed.
12. Incubate the microarray with the primary sample diluted in staining buffer overnight at 2 to 8 °C on an orbital shaker at 140 rpm. Dilute your primary sample as described in “Recommended Sample and Secondary Antibody Dilutions”.
13. Wash the peptide microarray 3x for one minute with washing buffer using an orbital shaker at 140 rpm, protected from light. Completely aspirate and remove the buffer. Prepare the secondary antibody solution for step 14.
14. Stain with secondary antibody diluted in staining buffer for 45 minutes at room temperature using an orbital shaker at 140 rpm, protected from light. Dilute the secondary antibody as described in “Recommended Sample and Secondary Antibody Dilutions”. Completely aspirate and remove the antibody solution.
15. Wash the peptide microarray 3x for one minute with washing buffer using an orbital shaker at 140 rpm, protected from light. Completely aspirate and remove the buffer.
16. Disassemble the incubation tray and carefully take out the microarray from the incubation chamber.
17. Directly dip the microarray 2 - 3 times into dipping buffer filled in a beaker. If necessary, slightly tilt the beaker. Repeat this step until all visible contaminations (e.g. PBS residues or dust) are removed.
18. Carefully dry the microarray completely in a pressurized air stream from top to bottom.
19. Scan the peptide microarray according to guidelines of your scanner using the appropriate fluorescence channels.

2-2 Experiment Method

GENE ON BIOTECH

Identification of the epitope of OOO by using OOO peptide array platform.

To identify the epitope of OOO antibody, OOO peptide array was produced followed by amino acids sequences that consist of OOO.
Three isoforms of the OOO sequences are synthesized with...

... .

3-1 Sample Optimization

GENE ON BIOTECH

Summary

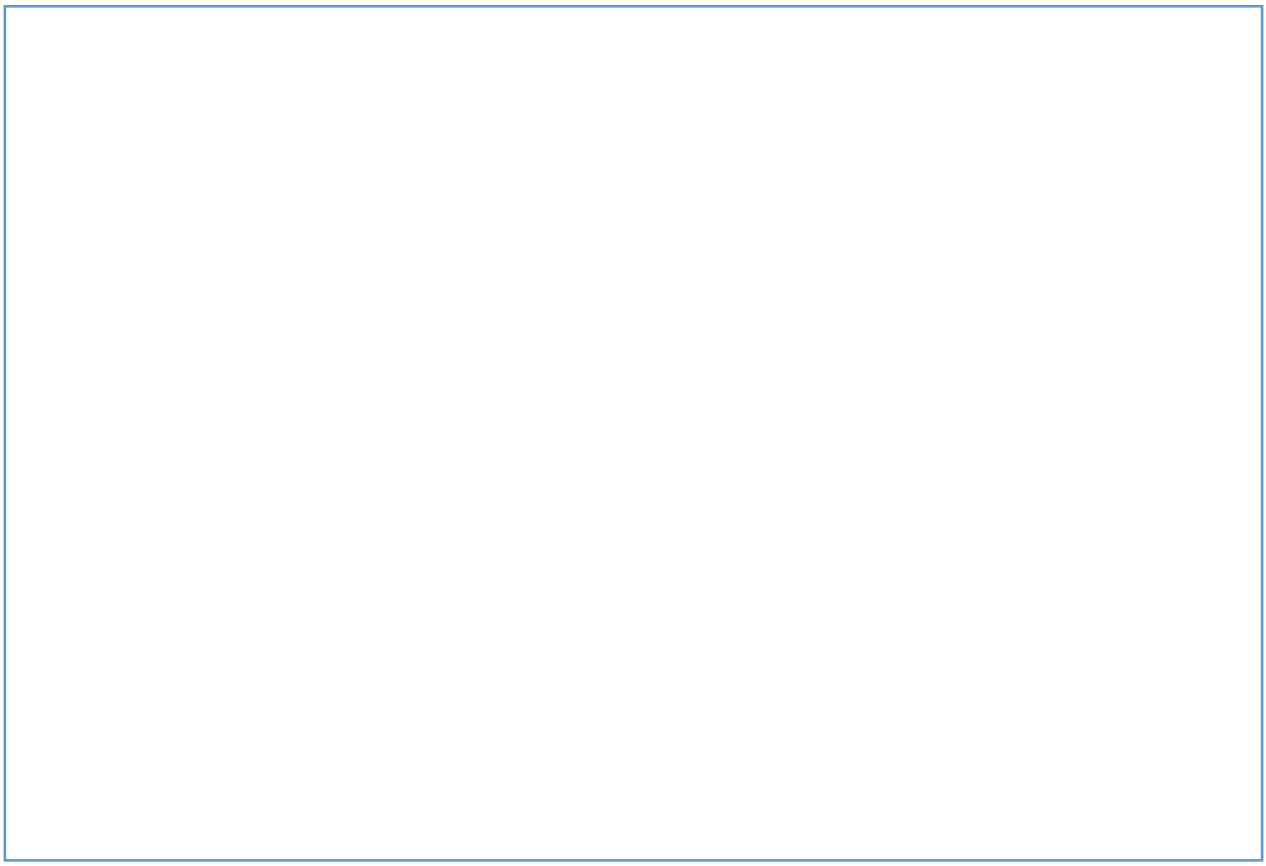
· OOO에 대한 테스트 진행

Result

· OOO OOO OOO

· 샘플의 전처리를 확인한 뒤 실험을 진행함.

Results image



3-2 Antigen information

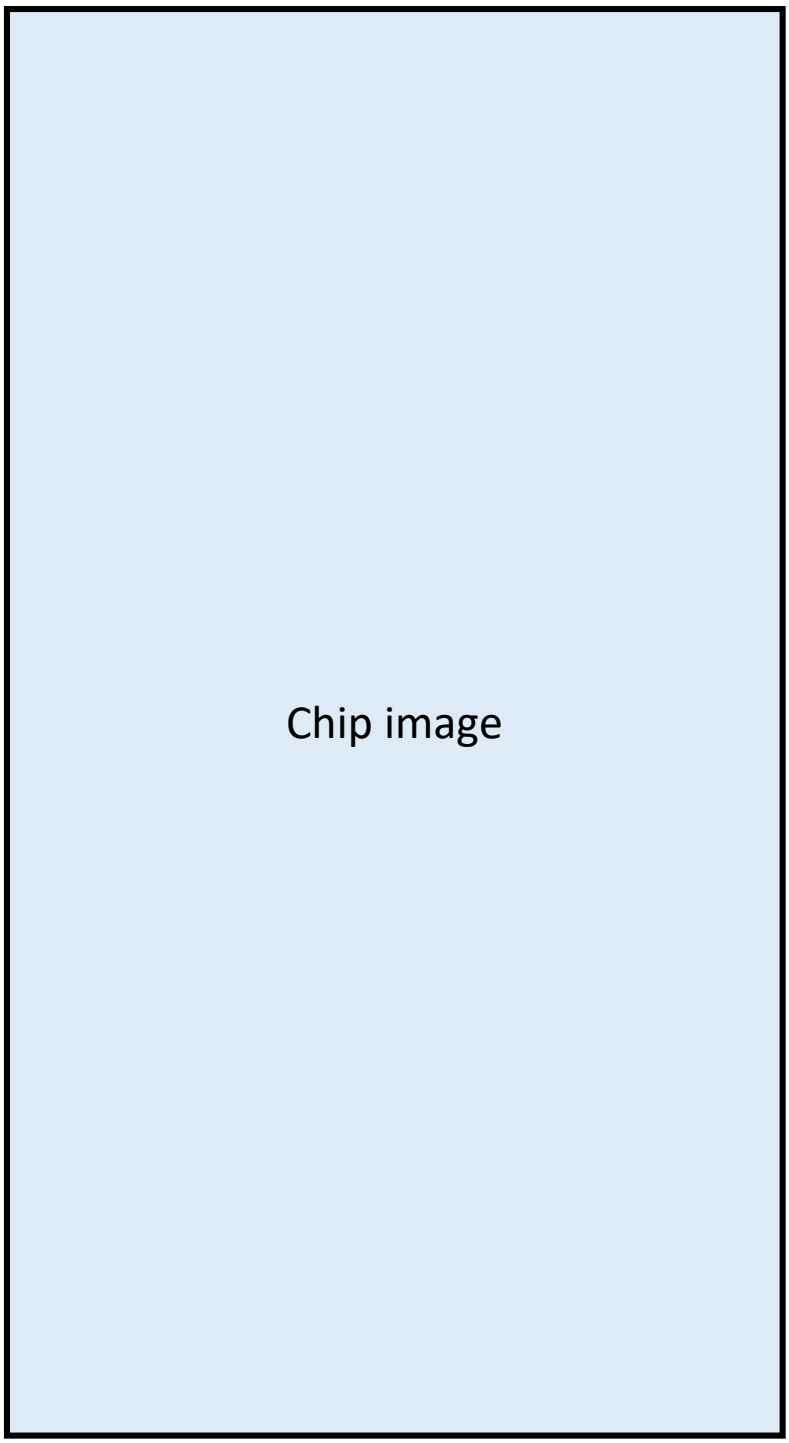
Protein information

Gene Title	**EGFR**
Protein	**Epidermal growth factor receptor**
Molecular function	ATP binding Chromatin binding Double-stranded DNA binding Enzyme binding Epidermal growth factor-activated receptor activity Epidermal growth factor binding
Biological process	Branching morphogenesis of an epithelial tube Cell surface receptor signaling pathway Endothelial cell morphogenesis Entry of bacterium into host cell Establishment of skin barrier Liver development MAPK cascade Multicellular organism development Negative regulation of autophagy Negative regulation of guanyl-nucleotide exchange factor activity Negative regulation of Rho protein signal transduction Negative regulation of stress fiber assembly Negative regulation of thrombin-activated receptor signaling pathway Neuron differentiation Pancreas development Positive chemotaxis Positive regulation of endothelial cell chemotaxis Semaphorin-plexin signaling pathway Signal transduction Transmembrane receptor protein tyrosine kinase signaling pathway
Cellular location	Cell membrane, Endoplasmic reticulum, Endosome, Golgi apparatus, Membrane, Nucleus, Secreted
Sequence	**Isoform 1**, **Isoform 2**, **Isoform 3**, **Isoform 4**
Additional information	UniProt: P08581 CCDS: CCDS43636 RefSeq: NM_000245 Ensembl: ENSP00000410980

4-1 Experiment: 결과 개요

GENE ON BIOTECH

Peptide array Experiment
Result image



Summary

000 000 000

Result

- 000 000 000

000 000 000

- 000 000 000000 000 000

분석 결과

000 000 000

4-1 Experiment: 결합 정보

GENE ON BIOTECH

Block	Column	Row	ID	Score
3	29	61	OOOOOOOOOO	6866
9	18	14	OOOOOOOOOO	2903.5
12	23	10	OOOOOOOOOO	3924.75
1	27	91	OOOOOOOOOO	4859.75
17	26	49	OOOOOOOOOO	2663.75

결합 펩타이드 중 반복서열 정보:

OOOOOOOOOOOOOOO
OOOOOOOOOOOOOOO
OOOOOOOOOOOOOOO
OOOOOOOOOOOOOOO
OOOOOOOOOOOOOOO

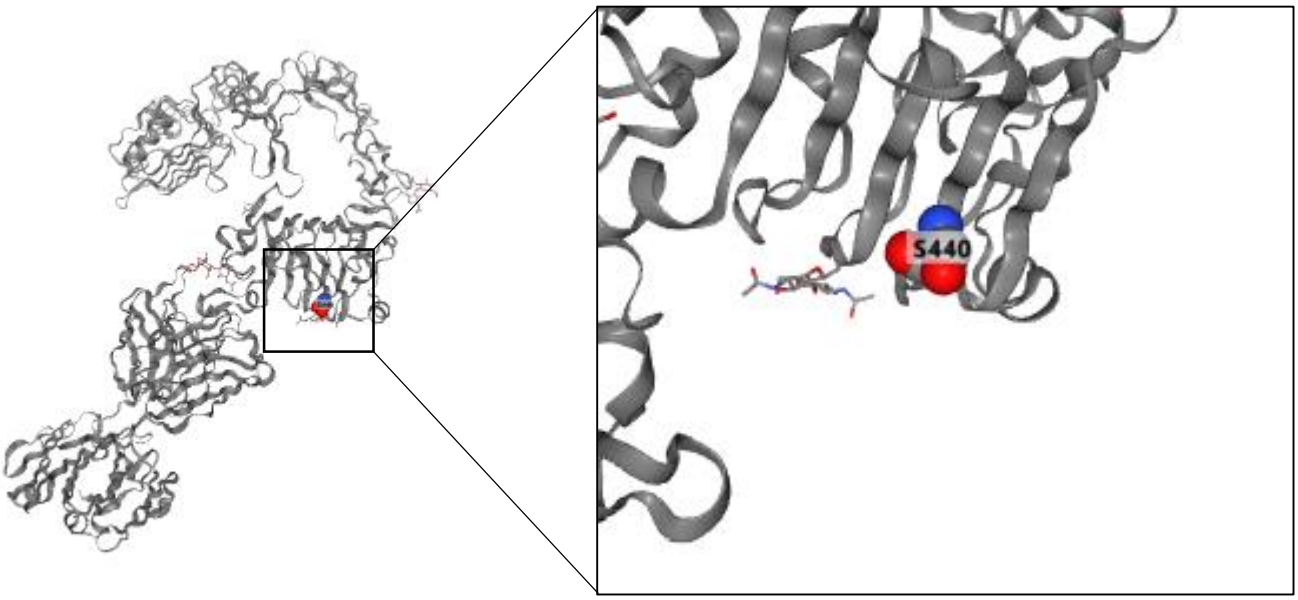
4-2 Experiment: 전체 서열 상 반복서열 위치

GENE ON BIOTECH

```
EGFR        MRPSGTAGAALLALLAALCPASRALEEKKVCQGTSNKLTQLGTFEDHFLSLQRMFNNCEV    60
Epitope     ------------------------------------------------------------    0

EGFR        VLGNLEITYVQRNYDLSFLKTIQEVAGYVLIALNTVERIPLENLQIIRGNMYYENSYALA    120
Epitope     ------------------------------------------------------------    0

EGFR        VLSNYDANKTGLKELPMRNLQEILHGAVRFSNNPALCNVESIQWRDIVSSDFLSNMSMDF    180
Epitope     ------------------------------------------------------------    0

EGFR        QNHLGSCQKCDPSCPNGSCWGAGEENCQKLTKIICAQQCSGRCRGKSPSDCCHNQCAAGC    240
Epitope     ------------------------------------------------------------    0

EGFR        TGPRESDCLVCRKFRDEATCKDTCPPLMLYNPTTYQMDVNPEGKYSFGATCVKKCPRNYV    300
Epitope     ------------------------------------------------------------    0

EGFR        VTDHGSCVRACGADSYEMEEDGVRKCKKCEGPCRKVCNGIGIGEFKDSLSINATNIKHFK    360
Epitope     ------------------------------------------------------------    0

EGFR        NCTSISGDLHILPVAFRGDSFTHTPPLDPQELDILKTVKEITGFLLIQAWPENRTDLHAF    420
Epitope     ------------------------------------------------------------    0

EGFR        ENLEIIRGRTKQHGQFSLAVVSLNITSLGLRSLKEISDGDVIISGNKNLCYANTINWKKL    480
Epitope     ------------------------------------------------------------    0

EGFR        FGTSGQKTKIISNRGENSCKATGQVCHALCSPEGCWGPEPRDCVSCRNVSRGRECVDKCN    540
Epitope     ------------------------------------------------------------    0

EGFR        LLEGEPREFVENSECIQCHPECLPQAMNITCTGRGPDNCIQCAHYIDGPHCVKTCPAGVM    600
Epitope     ------------------------------------------------------------    0

EGFR        GENNTLVWKYADAGHVCHLCHPNCTYGCTGPGLEGCPTNGPKIPSIATGMVGALLLLLVV    660
Epitope     ------------------------------------------------------------    0

EGFR        ALGIGLFMRRRHIVRKRTLRRLLQERELVEPLTPSGEAPNQALLRILKETEFKKIKVLGS    720
Epitope     ------------------------------------------------------------    0

EGFR        GAFGTVYKGLWIPEGEKVKIPVAIKELREATSPKANKEILDEAYVMASVDNPHVCRLLGI    780
Epitope     ------------------------------------------------------------    0

EGFR        CLTSTVQLITQLMPFGCLLDYVREHKDNIGSQYLLNWCVQIAKGMNYLEDRRLVHRDLAA    840
Epitope     ------------------------------------------------------------    0

EGFR        RNVLVKTPQHVKITDFGLAKLLGAEEKEYHAEGGKVPIKWMALESILHRIYTHQSDVWSY    900
Epitope     ------------------------------------------------------------    0

EGFR        GVTVWELMTFGSKPYDGIPASEISSILEKGERLPQPPICTIDVYMIMVKCWMIDADSRPK    960
Epitope     ----------------------SSILEKGERLPQPPIC----------------------    16
                                   ****************
EGFR        FRELIIEFSKMARDPQRYLVIQGDERMHLPSPTDSNFYRALMDEEDMDDVVDADEYLIPQ    1020
Epitope     ------------------------------------------------------------    16

EGFR        QGFFSSPSTSRTPLLSSLSATSNNSTVACIDRNGLQSCPIKEDSFLQRYSSDPTGALTED    1080
Epitope     ------------------------------------------------------------    16

EGFR        SIDDTFLPVPEYINQSVPKRPAGSVQNPVYHNQPLNPAPSRDPHYQDPHSTAVGNPEYLN    1140
Epitope     ------------------------------------------------------------    16

EGFR        TVQPTCVNSTFDSPAHWAQKGSHQISLDNPDYQQDFFPKEAKPNGIFKGSTAENAEYLRV    1200
Epitope     ------------------------------------------------------------    16

EGFR        APQSSEFIGA          1210
Epitope     ----------          16
```

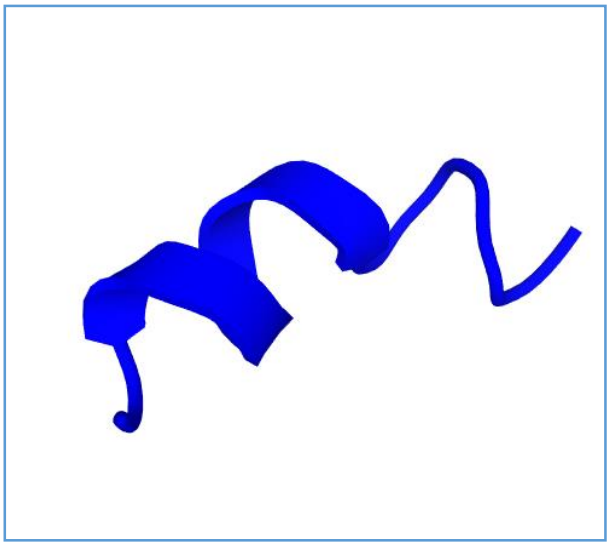
4-2 Experiment: 전체 서열 상 반복서열 위치

RTKQHGQFSLAVV(440-452)



4-3 Experiment: 서열 상세정보

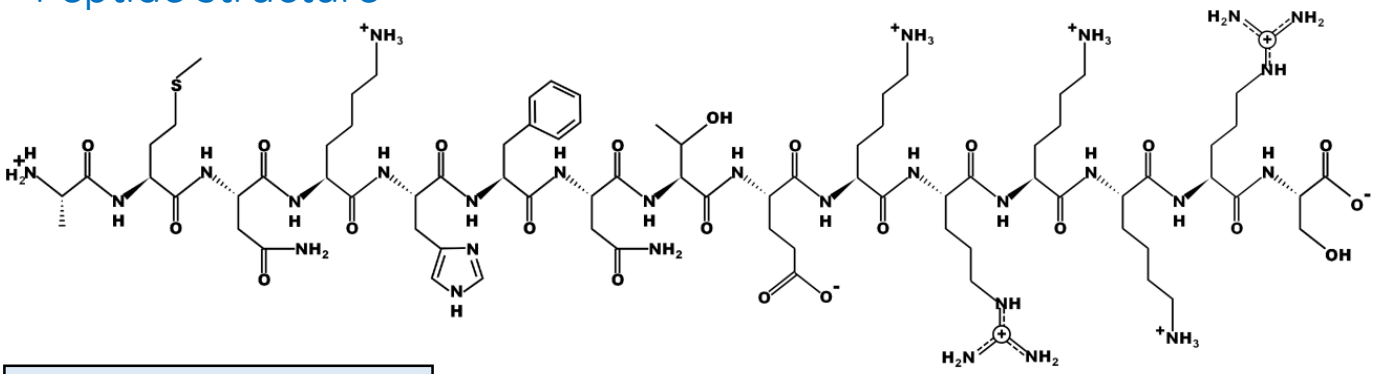
서열정보: AEKOOHFNTNRS



Peptide properties

Sequence	**AEKOOHFNTNRS**
Length	15
Mass	1874.0029
Isoelectric point (pI)	11.69
Net charge	+5
Hydrophobicity	+29.21 Kcal * mol $^{-1}$
Extinction coefficient	0 M^{-1} * cm^{-1}

Peptide structure



Chip image

Signal image