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Supporting Information

Supplementary Methods

The titer analysis of oyster AK polyclonal antibodies

The oyster AK polyclonal antibody titer was measured by dot blot and ELISA, following the method of Liu et al.¹ The specific polyclonal antibody (1:500,000 dilution) was then recruited as the primary antibody for the subsequent experiments.

Analysis of physical and chemical properties of AK

Native PAGE analysis of AK is performed according to the method of He et al.² The isoelectric point of AK was predicted by ExPASy online server. The disulfide bonds were predicted by bioinformatics sites (http://www.csbio.sjtu.edu.cn/bioinf/Cyscon/).

References

- 1. Y. Y. Liu, M. J. Cao, M. L. Zhang, J. W. Hu, Y. X. Zhang, L. J. Zhang, G. M. Liu, Purification, characterization and immunoreactivity of β'-component, a major allergen from the roe of large yellow croaker (*Pseudosciaena crocea*). *Food Chem Toxicol*. 2014, **72**, 111-121.
- 2. X. R. He, Y. M. Cheng, Y. Yang, J. J. Xie, K. H. Chu, Y. X. Zhang, G. X. Chen, H. Liu, M. J. Cao, G. M. Liu, Cloning, expression and comparison of the properties of Scy p 9, a *Scylla paramamosain* allergen, *Food Funct.*, 2020, **11**(4), 3006-3019.

Supplementary Figure Legends

Figure S1. Determination of the oyster AK polyclonal antibody titer.

- (A) ELISA analysis of the AK polyclonal antibody titers, BSA was used as a negative control. All data were presented as the mean \pm SD (n = 3).
- (B) Dot blot analysis of the AK polyclonal antibody titers. 1:10³ -1:10⁶ is the polyclonal antibody dilution.

Figure S2. Analysis of physicochemical properties of AK.

- (A) Native PAGE analysis of AK.
- (B) Western blot analysis of AK after Native PAGE using oyster AK polyclonal antibody (1:500,000).
- (C) Theoretical isoelectric point prediction by ExPASy online server.
- (D-E) Disulfide bonds were predicted by bioinformatics sites and displayed in 3D model.

Table S1. AK epitopes predicted by five immunoinformatic tools.

Software	Epitope position
DNA star	5-27, 30-42, 84-92, 96-101, 121-125, 130-145, 151-159, 165-176, 189-200, 227-234, 281-289, 307-313, 318-323
BepiPred 1.0 server	12-17, 32-39, 59-69, 86-108, 121-143, 165-174, 186-199, 228-234, 306-317
ABCpred Server	11-26, 62-109, 121-146, 157-172, 180-210, 248-263, 302-341
Immunomedicine Group	16-30, 40-54, 56-95, 110-120, 125-133, 231-266, 272-282, 310-317
NetMHC II 2.3 Server	68-82, 141-159, 219-246

Table S2. Ten epitopes were selected from five immunoinformatic tools.

Number	Amino acid sequence	Position	Length	Identified epitope
P1	LSEGESKSLLKKHCTKE	11-27	17	-
P2	SGAGVYACDPEGYEVFKE	56-73	18	L-AK-1
Р3	PVIMDYHKVDKVEHPP	77-92	16	L-AK-2
P4	CDFGPQDKLGFDPLD	93-107	15	L-AK-3
P5	GRSHEGYPFPPVS	121-133	13	L-AK-4
P6	TDEQRKEMENKTI	134-146	13	L-AK-5
P7	TMTPEENQQLID	165-176	12	L-AK-6
P8	DKMLGDAGGYNGWPKA	185-200	16	L-AK-7
P9	QKGGDVGEVYKR	227-238	12	L-AK-8
P10	GEHTESVGGVYDISNKRR	306-323	18	L-AK-9

Figure S1

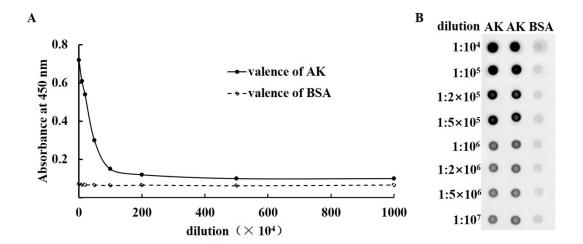
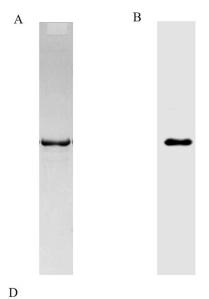


Figure S2



Query name: AK

Protein sequence:

MSDLPELWKKLSEGESKSLLKKHCTKEVYEKLKDKKTKLGGTLADCIRSG ALNLNSGAGVYACDPEGYEVFKELLDPVIMDYHKVDKVEHPPCDFGPQDK LGFDPLDATGEFIVSTRVRVGRSHEGYPFPVSTDEQRKEMENKTIAALN TLEGELKGKYYSLETMTPEENQQLIDDHFMFKNDDKMLGDAGGYNGWPKA RGIFFNDNKTFLCWINEEDHLRFISMQKGGDVGEVYKRLVSAIKQLEKKL TFAYSKRHGYLTFCPTNLGTTLRASVHIKVPKLAKNEEVLNKICADNKLQ LRGIHGEHTESVGGVYDISNKRRLGLTEYQAMQEMYNGIKEIIQAEKNAK

Predicted disulfide bonds:

SSBOND#1: 46-63 SSBOND#2: 264-294

ProtParam

User-provided sequence:

1 <u>0</u> MSDLPELWKK	2 <u>0</u> LSEGESKSLL	3 <u>0</u> KKHCTKEVYE	4 <u>0</u> KLKDKKTKLG	5 <u>0</u> GTLADCIRSG	6 <u>0</u> ALNLNSGAGV
7 <u>0</u> YACDPEGYEV	8 <u>0</u> FKELLDPVIM		10 <u>0</u> PPCDFGPQDK		12 <u>0</u> EFIVSTRVRV
	14 <u>0</u> PVSTDEQRKE				18 <u>0</u> NQQLIDDHFM
	20 <u>0</u> AGGYNGWPKA				24 <u>0</u> DVGEVYKRLV
25 <u>0</u> SAIKQLEKKL	26 <u>0</u> TFAYSKRHGY		28 <u>0</u> TLRASVHIKV		30 <u>0</u> NKICADNKLQ
31 <u>0</u> LRGIHGEHTE	32 <u>0</u> SVGGVYDISN		34 <u>0</u> AMQEMYNGIK		

References and documentation are available.

Number of amino acids: 350

Theoretical pI: 6.58

