

Multiplex Detection of 7 Staphylococcal Enterotoxins to use liquid chromatography-mass spectrometry combined with a novel capture molecule

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Table S1 Specific peptide sequence

Peptide	sequence	Molecular Weight[Da]	Retention time
SEA3	QNTVPLETVK	1128.291	2.56
SEA4	NVTVQELDLQAR	1385.54	3.20
SEA3R*	QNTVPLETV	1136.0	2.56
SEB2	VLYDDNHVSAINVK	1586.765	2.68
SEB4	LGNYDNVR	950.019	1.81
SEB6	VTAQELDYLTR	1308.454	3.33
SEB6R*	VTAQELDYL	1318.2	3.32
SEC1	VLYDDHYVSATK	1410.546	2.29
SEC2	TELLNEGLAK	1087.238	2.91
SEC3R*	FLAHDLIYNISD	1556.4	3.72
SED4	NVDVYPIR	975.112	2.86
SED5	LYNNDTLGGK	1094.189	1.99
SED5R*	LYNNDTLGG	1102.2	1.99
SEE1	NALSNLR	786.886	2.28
SEE4	QTTVPIDK	901.028	1.90
SEE4R*	QTTVPID	908.8	1.90
SEH1	SDEISGEK	863.877	1.01
SEH3	FATADLAQK	964.086	2.32
SEH5R*	NVTLQELDI	1179.8	3.52
SEG2	TELENTELANNYK	1538.631	2.50
SEG4	NMVTIQELDYG	1353.553	3.45
SEG6	FLNIYGDNK	1083.209	3.21

Table S2: Peptide profile match rate of protein sequence

Protein	SEA	SEB	SEC	SED	SEE	SEG	SEH
Match coverage	74%	93%	93%	83%	99%	99%	99%

Table S3: Target protein information

Protein	SEA	SEB	SEC	SED	SEE	SEG	SEH	MHCII	MHCIID10
Molecular Weight[kDa]	28	29	28	27	27	28	26	23	37
Concentration (mg/mL)	0.61	1.32	1.14	0.67	1.47	1.23	1.62	1.48	0.54
yield (%)	4.17	8.58	6.84	5.16	12.7	14.0	9.72	8.61	1.62
purification-fold	14.6	15.4	16.7	13.0	11.6	8.8	16.7	17.2	33.3

a)

起始密码子
↓
MGMSDKIIHLTDDSFDTDLKADGAILVDFWAEWCGPCKMIAPILDEIADEYQGKLTVAKLNIDQN
NcoI Trx-tag
PGTAPKYGIRGIPTLLLKNGEVAATKVGALSKGQLKEFLDANLAGGGGSGGGGSGGGGS
linker
IKEEHV I I QAEFYLNPDQSGEFMFDFDGDEI FHVDMAKKETVWRLEEFGR
MHC
FASFEAQGALANIAVDKANLEIMTKRSNYTP ITN HHHHHH*
His tag XcoI

b)

起始密码子
↓
MGMSDKIIHLTDDSFDTDLKADGAILVDFWAEWCGPCKMIAPILDEIADEYQGKLTVAKLNIDQNP
NcoI Trx-tag
GTAPKYGIRGIPTLLLKNGEVAATKVGALSKGQLKEFLDANLAGGGGSGGGGSGGGGS
linker
IKEEHV I I QAEFYLNPDQSGEFMFDFDGDEI FHVDMAKKETVWRLEEFGRFASFEAQ
MHC
GALANIAVDKANLEIMTKRSNYTP ITN GGGGSGGGGSGGGGSGAVVSQHP SMVIVKSG
linker
TSVKIECRSLDTNIHTMFWRQFPKQSLMLMATSHQG FNAIYEQGVVKDKFLINHAS
TCR-D10
PTLSTLTVTSAHPEDSGFYVCSALAGSGSSTDTQYFGPGTQLTVL HHHHHH*
His tag XcoI

Figure S1 Sequence diagrams of the capture molecules MHCII and MHCII-D10

a) The capture molecule MHCII sequence b) The capture molecule MHCII-D10 sequence

* means a termination codon

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SEA SERSEEINERIRKSEIQG..TALNLRQIYYNEKARTENKESHDQFIQHTILFRGFTDHSWYNDLIVDFDSKDIVKYGSRNIIYGAYGVCAGGTPN.....RTAGVGGTTHDNNRLTE..EKKVPIINLIDGKQN
SEB ESQPDFPDPHRSKFT...GLNEMKVIDD.CNHVSAINVKSIDQFIYFLLYSIKCTKLGNYDNVVEFKNRDLAKYKGMVYVFGANVYCCYFSKRTNDINSHQITKRRKTMMGGSEHNGNQLR..YRSITVRVFEDGRNL
SEC1 ESQPDFPDPHRSKFT...GLNEMKVIDD.CHYVSATKVSVDKFLAHLIYINISCKRLKNYDKVTELLNEGLAKYKGMVYVFGANVYCCYFSKSD...NVGKVTGGKTMMGGSEHNGNQLR..YRSITVRVFEDGRNL
SEC2 NENIDSVKEKDPHRSKFT...TALNMRHSACKNEIIGENKSTGDOFENTLLFRGFTDHSWYNDLIVDFDSKDIVKYGSRNIIYGAYGVCAGGTPN.....RTAGVGGTTHDNNRLTE..EKKVPIINLIDGKQN
SEE SEEINERIRKSEIQR..NALNLRQIYYNEKARTENKESHDQFIQHTILFRGFTDHSWYNDLIVDFDSKDIVKYGSRNIIYGAYGVCAGGTPN.....RTAGVGGTTHDNNRLTE..EKKVPIINLIDGKQN
SEG QPDFKLDPHRSKFT...GLNEMKVIDD.CHYVSATKVSVDKFLAHLIYINISCKRLKNYDKVTELLNEGLAKYKGMVYVFGANVYCCYFSKSD...NVGKVTGGKTMMGGSEHNGNQLR..YRSITVRVFEDGRNL
SEH ETRHDSKELTD..LAINVAYGON..HFKENIKSDEISGEKDLIFR...NQGDSGNDLRVKRATADLACKRNNNIIYGASFYYPGEISEN.....ISEHGGTTHN.SEKLAQ..ERVIGANVWVDGIGK
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Figure S2 Sequence alignment map of the SEs proteins

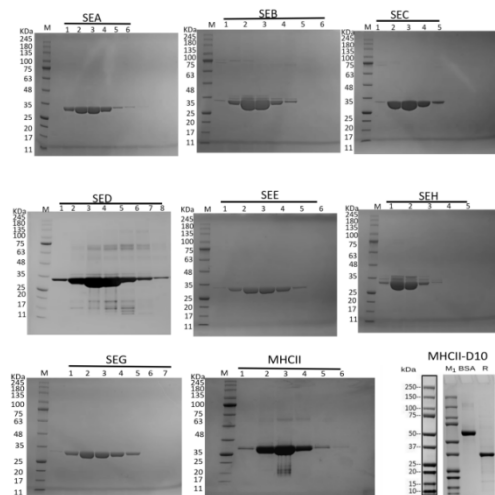
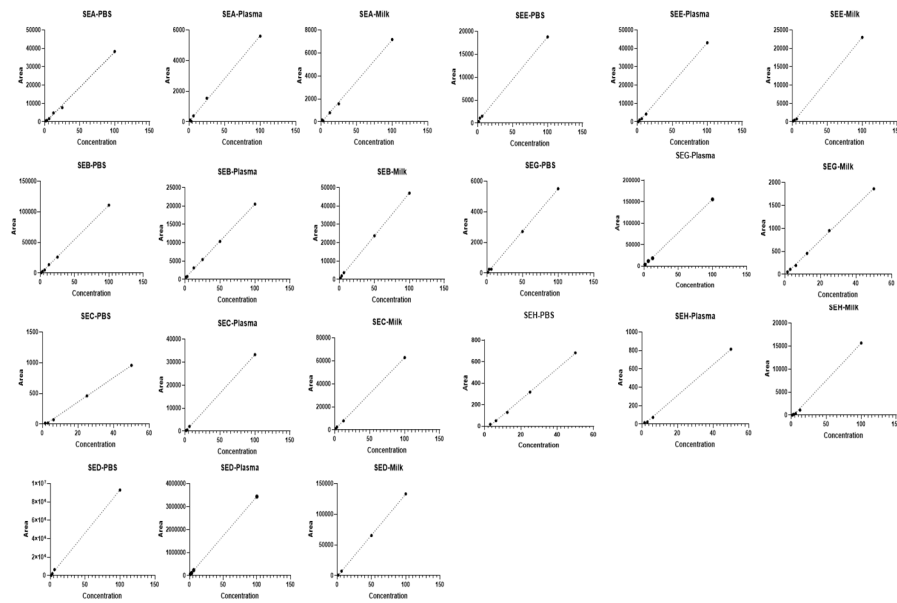


Figure S3. Toxin and capture molecule gel electrophoresis

a)



b)

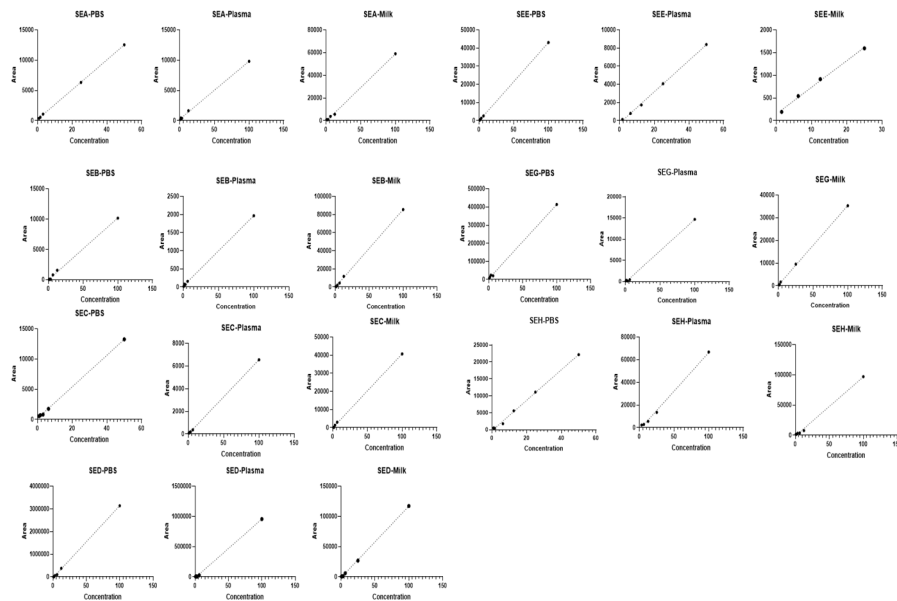


Figure S4: Standard curves of 7 serotypes of toxins in 3 different substrates of MHCII and MHCII-D10 capture molecule, 1xPBS, milk and plasma were used

a) Standard curves of 7 serotypes of toxins in 3 different substrates including MHCII capture molecule, 1xPBS milk and plasam were used. **b)** Standard curves of 7 serotypes of toxins in 3 different substrates including MHCII-D10 capture molecule, 1xPBS milk and plasam were used.