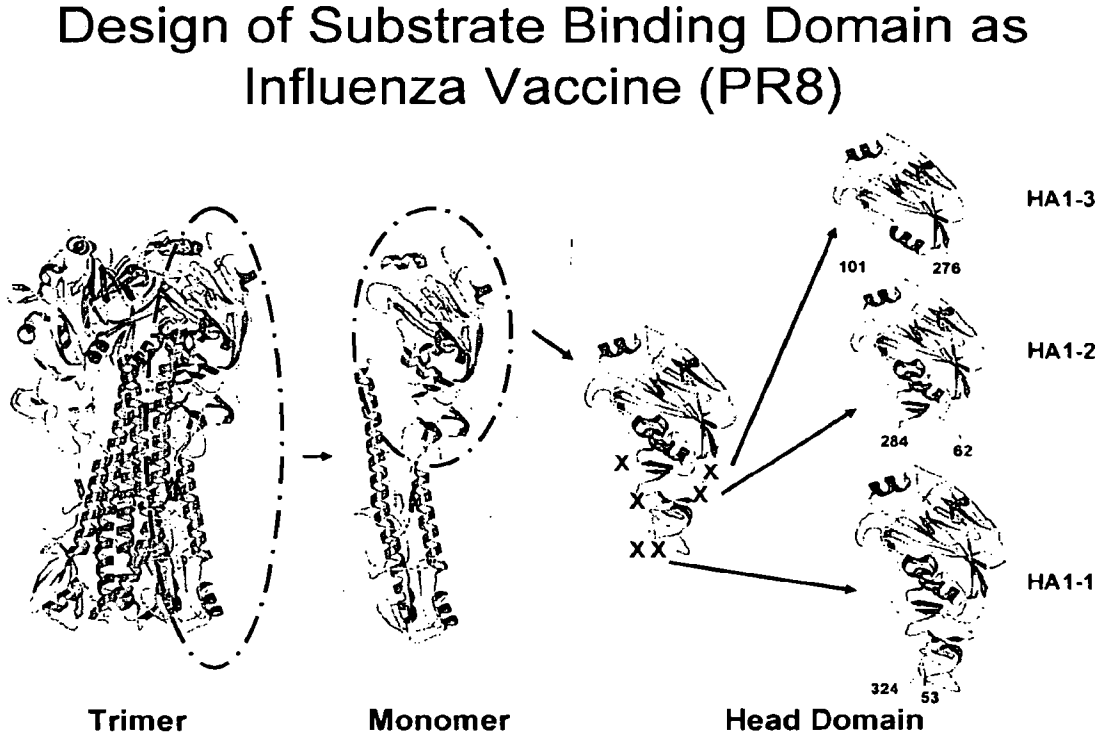

Design of Substrate Binding Domain as Influenza Vaccine (PR8)
HA1-3
HA1-2
HA1-1
101
276
284
62
324
53
Trimer
Monomer
Head Domain

Figure 1

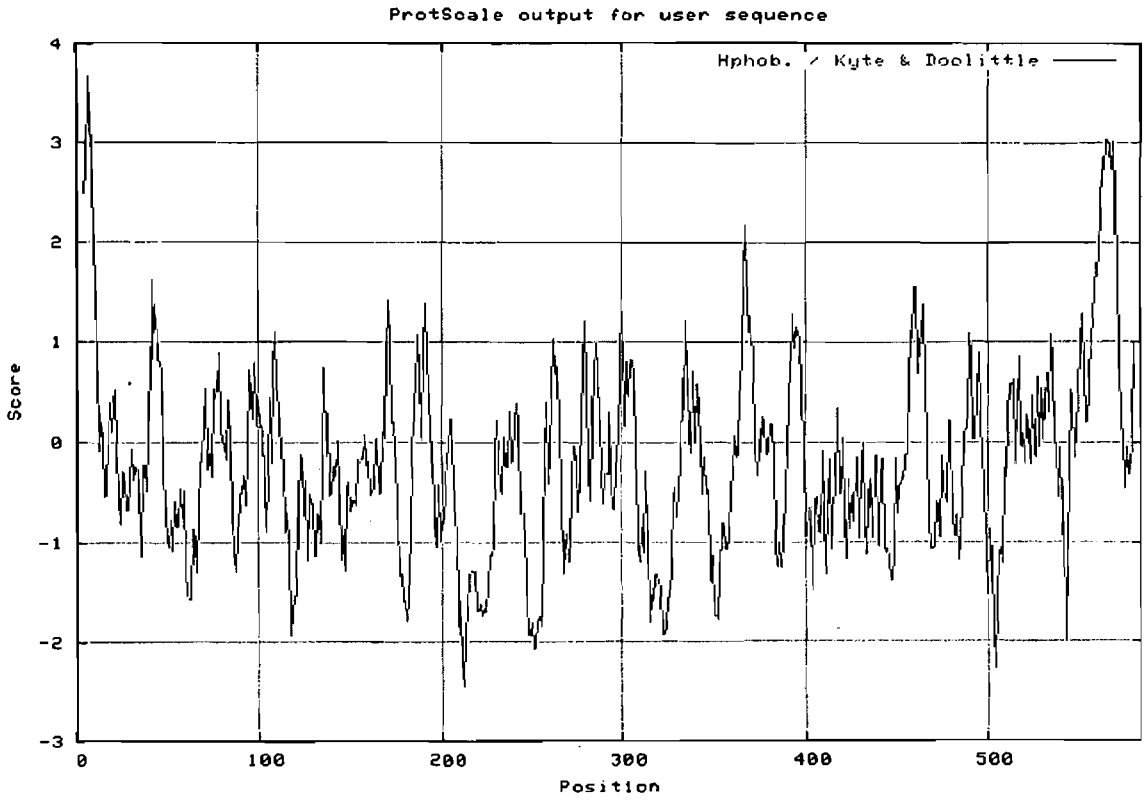

ProtScale output for user sequence
Hphob. / Kyte & Doolittle
Score
Position
4
3
2
1
0
-1
-2
-3
0
100
200
300
400
500

Figure 2

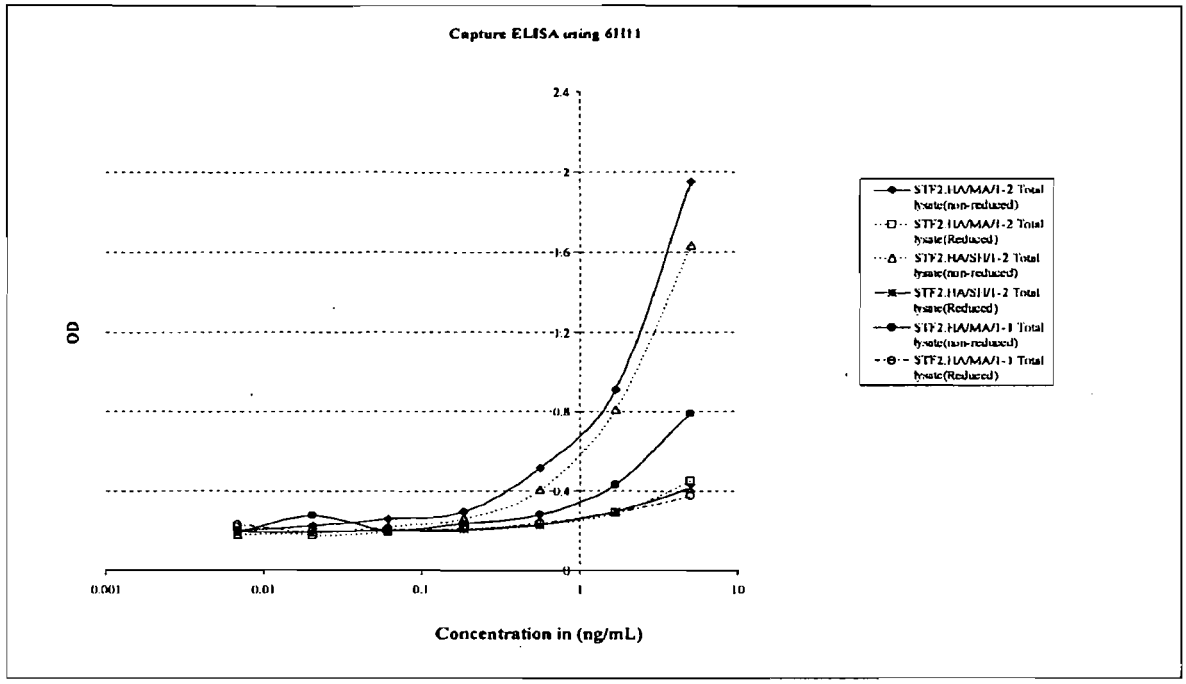
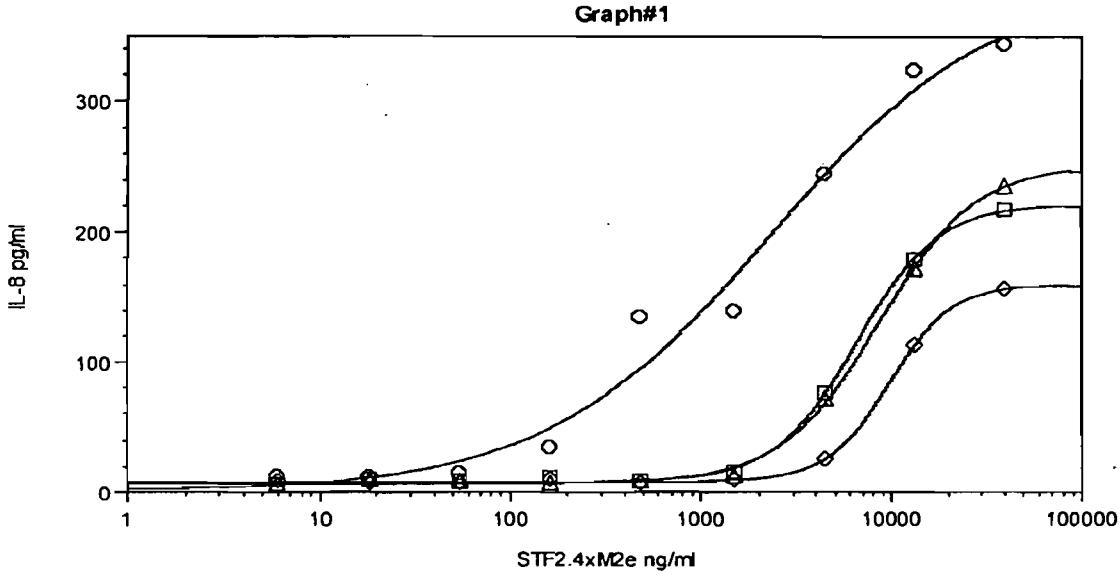

Capture ELISA using 61111
OD
2.4
2
1.6
1.2
0.8
0.4
0
0.001
0.01
0.1
1
10
Concentration in (ng/mL)
STF2.HA/MA/1-2 Total lysate(non-reduced)
STF2.HA/MA/1-2 Total lysate(Reduced)
STF2.HA/SH/1-2 Total lysate(non-reduced)
STF2.HA/SH/1-2 Total lysate(Reduced)
STF2.HA/MA/1-1 Total lysate(non-reduced)
STF2.HA/MA/1-1 Total lysate(Reduced)

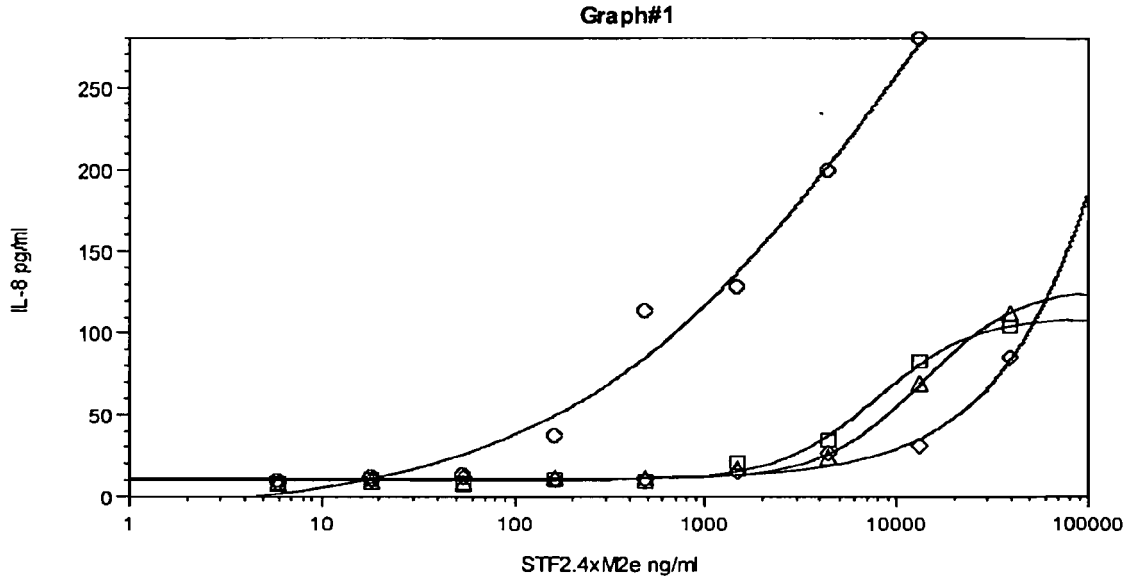
Figure 3



4-P Fit: y = (A - D)/(1 + (x/C)^B) + D:	A	B	C	D	R^2
○ Plot#1 (REF STF24xM2e@Experiment#1: Dilution vs ...	0.802	0.747	2256.726	391.104	0.98
□ Plot#2 (Mal 1-2 Pellet@Experiment#1: Dilution vs Mea...	8.074	2.026	6520.987	221.39	1
△ Plot#3 (SH 1-2 Pellet@Experiment#1: Dilution vs Mea...	6.638	1.686	8508.737	251.886	0.999
◇ Plot#4 (Mal 1-1 Pellet@Experiment#1: Dilution vs Mea...	8.141	2.621	9795.127	160.022	1

Curve Fit Option - Fixed Weight Value: 1

Figure 4



4-P Fit: y = (A - D)/(1 + (x/C)^B) + D:	A	B	C	D	R^2
○ Plot#1 (REF STF24xM2e@Experiment#1: Dilution vs ...	-11.075	0.501	10458.24	530.647	0.982
□ Plot#2 (Mal 1-2 Sup@Experiment#1: Dilution vs Mean...	9.743	1.712	8003.096	110.268	0.996
△ Plot#3 (SH 1-2 Sup@Experiment#1: Dilution vs Mean...	10.11	1.703	13620.02	128.77	0.997
◇ Plot#4 (Mal 1-1 Sup@Experiment#1: Dilution vs Mean...	10.599	1.047	5.86e5	1302.212	0.983

Curve Fit Option - Fixed Weight Value: 1

Figure 5

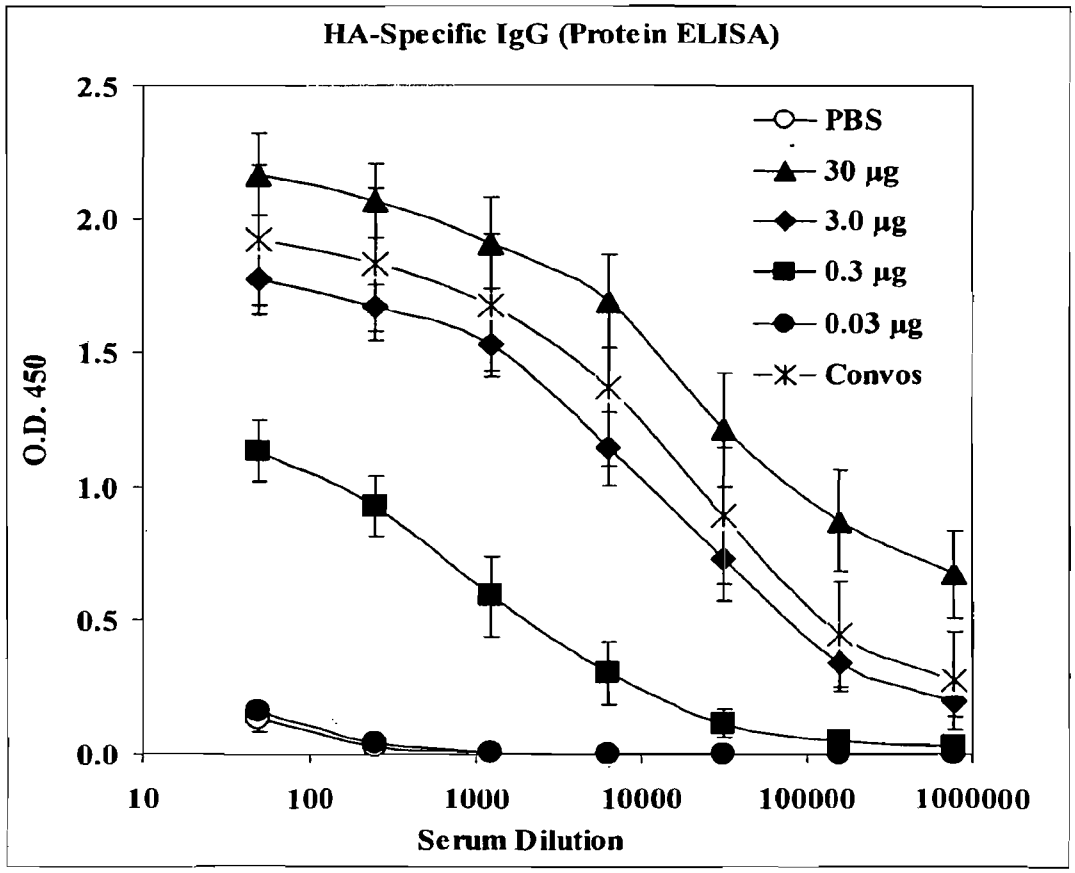

HA-Specific IgG (Protein ELISA)
2.5
2.0
1.5
1.0
0.5
0.0
O.D. 450
10
100
1000
10000
100000
1000000
Serum Dilution
PBS
30 µg
3.0 µg
0.3 µg
0.03 µg
Convos

Figure 6

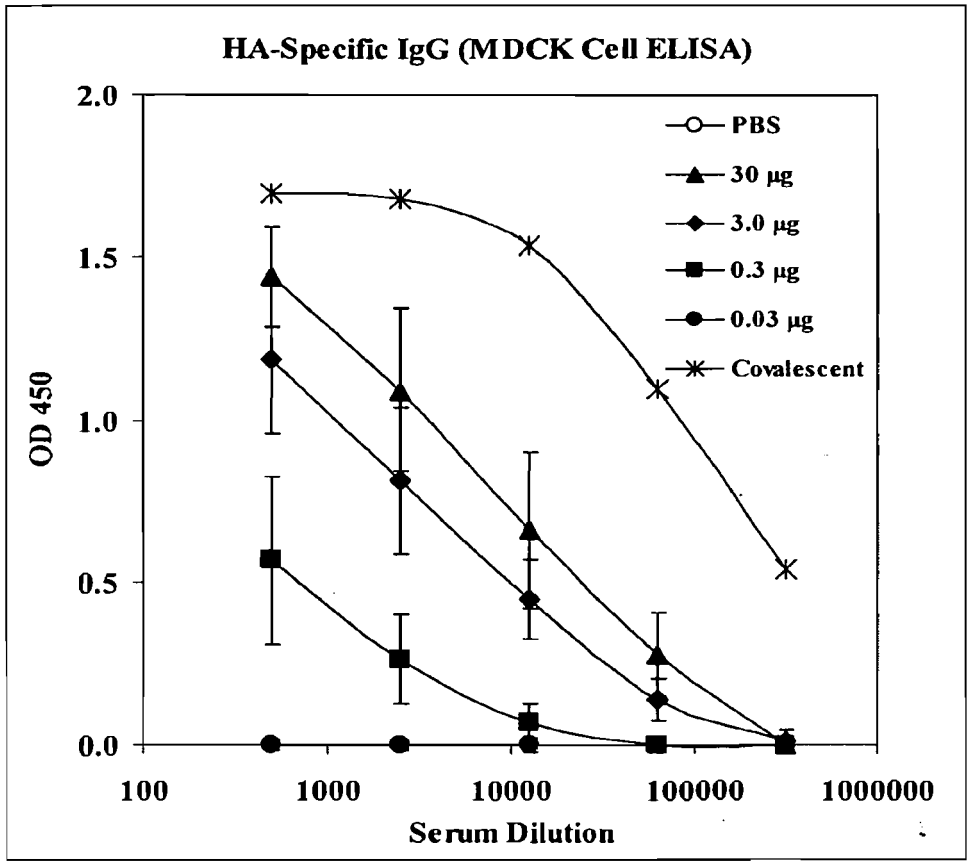

HA-Specific IgG (MDCK Cell ELISA)
2.0
1.5
1.0
0.5
0.0
OD 450
100
1000
10000
100000
1000000
Serum Dilution
PBS
30 µg
3.0 µg
0.3 µg
0.03 µg
Covalescent

Figure 7

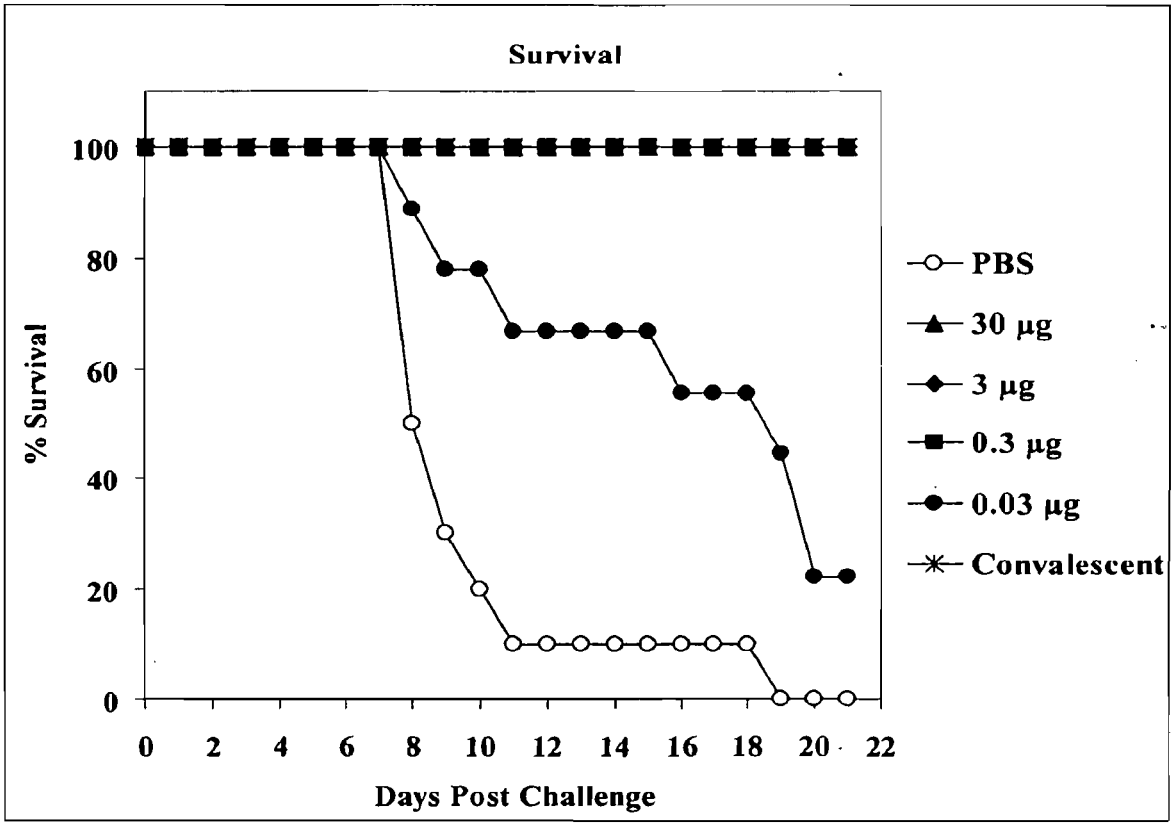

Survival
100
80
60
40
20
0
% Survival
0
2
4
6
8
10
12
14
16
18
20
22
Days Post Challenge
PBS
30 µg
3 µg
0.3 µg
0.03 µg
Convalescent

Figure 8A

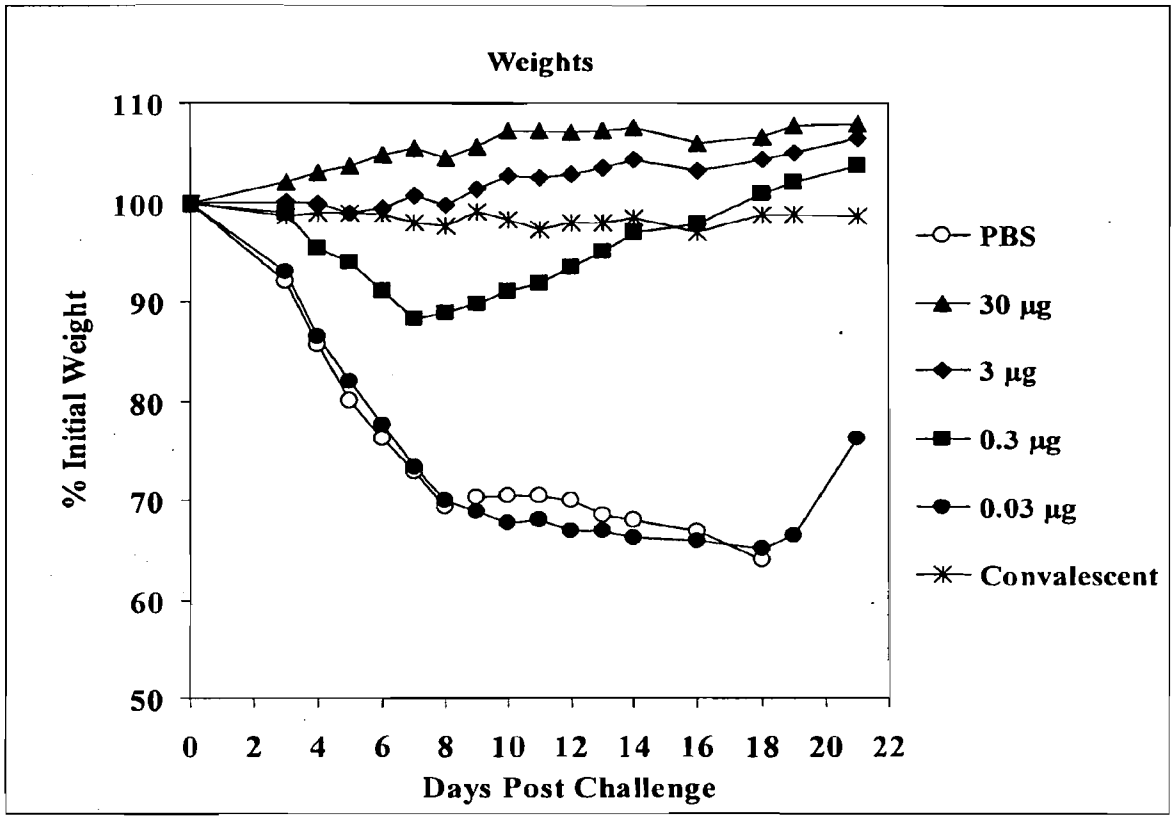

Weights
110
100
90
80
70
60
50
% Initial Weight
0
2
4
6
8
10
12
14
16
18
20
22
Days Post Challenge
PBS
30 µg
3 µg
0.3 µg
0.03 µg
Convalescent

Figure 8B

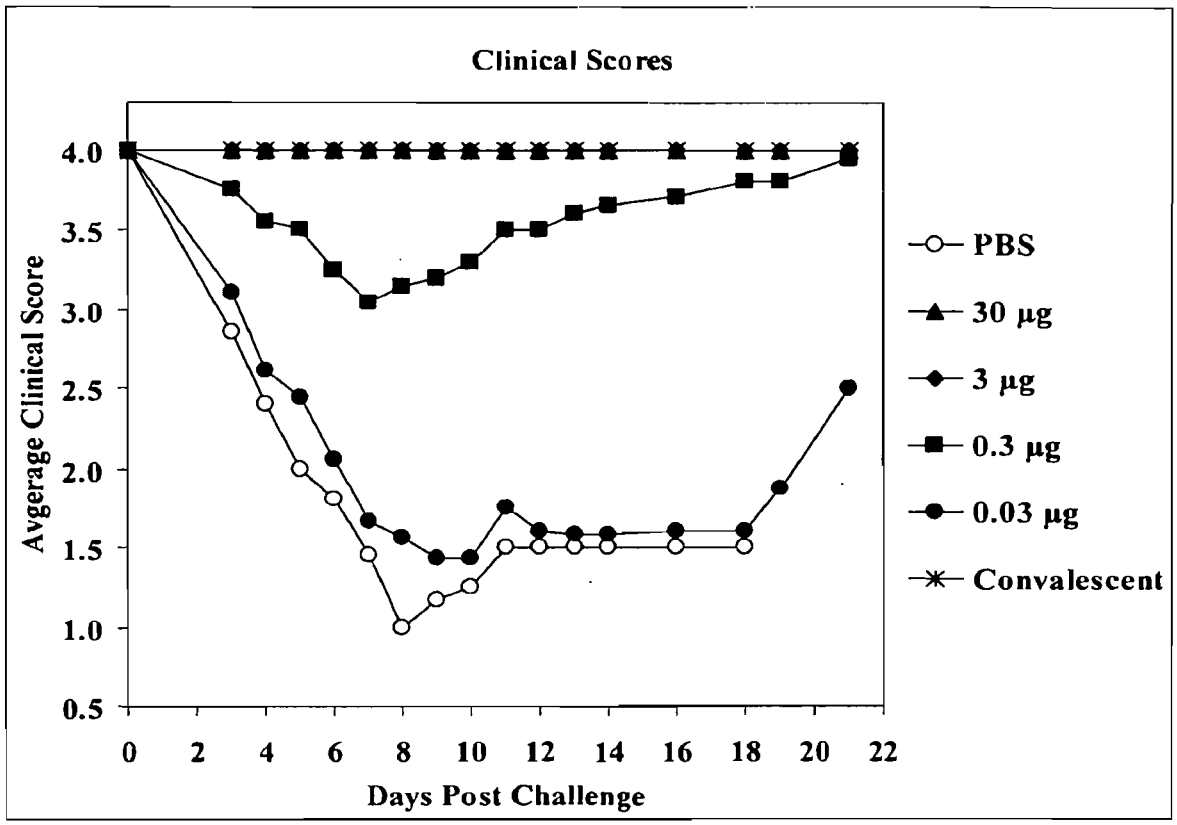

Clinical Scores
Avgerage Clinical Score
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0
2
4
6
8
10
12
14
16
18
20
22
Days Post Challenge
PBS
30 µg
3 µg
0.3 µg
0.03 µg
Convalescent

Figure 8C

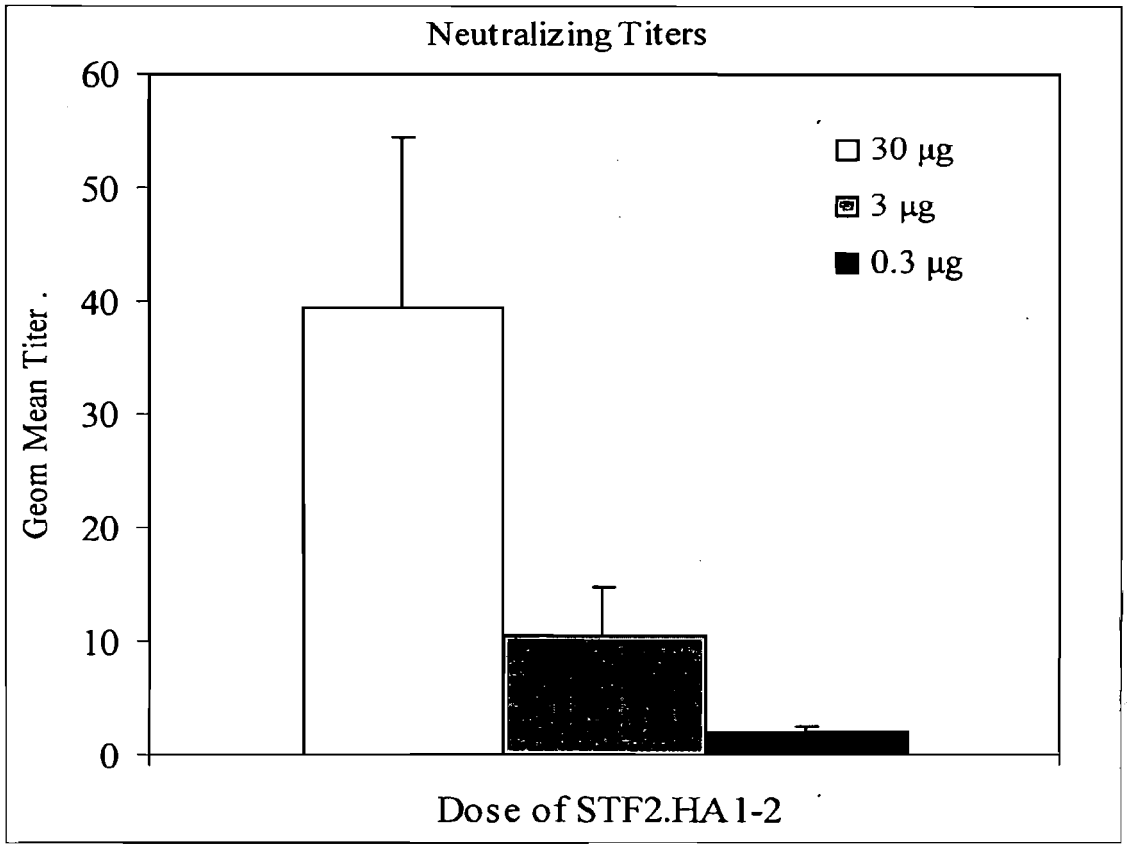

Neutralizing Titers
60
50
40
30
20
10
0
Geom Mean Titer .
30 µg
3 µg
0.3 µg
Dose of STF2.HA1-2

Figure 9

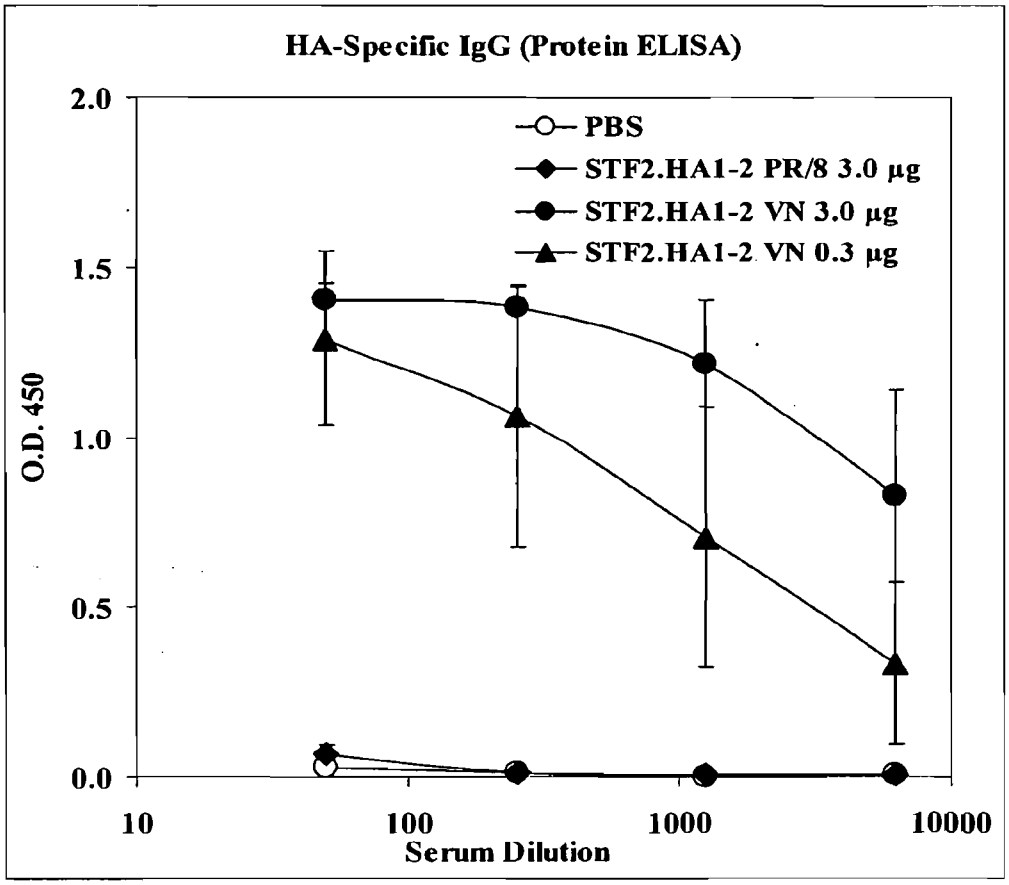

HA-Specific IgG (Protein ELISA)
2.0
1.5
1.0
0.5
0.0
O.D. 450
PBS
STF2.HA1-2 PR/8 3.0 µg
STF2.HA1-2 VN 3.0 µg
STF2.HA1-2 VN 0.3 µg
10
100
1000
10000
Serum Dilution

Figure 10A

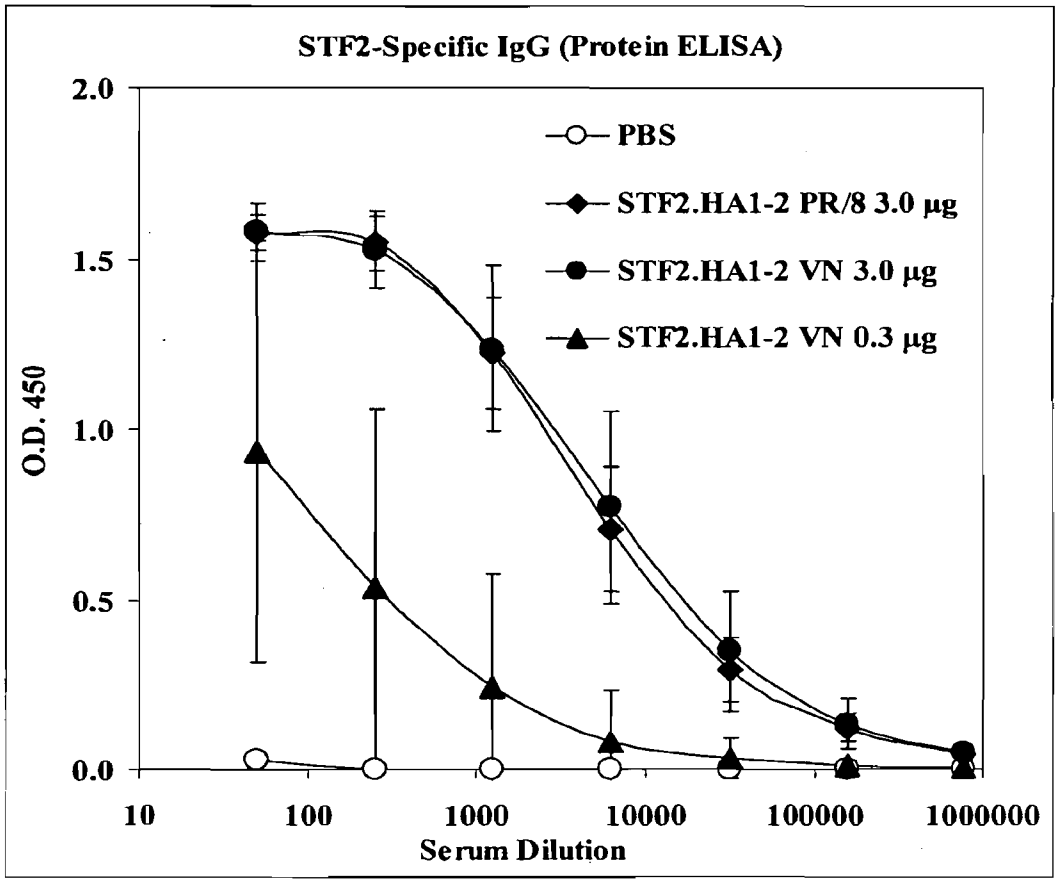

STF2-Specific IgG (Protein ELISA)
2.0
1.5
1.0
0.5
0.0
O.D. 450
PBS
STF2.HA1-2 PR/8 3.0 µg
STF2.HA1-2 VN 3.0 µg
STF2.HA1-2 VN 0.3 µg
10
100
1000
10000
100000
1000000
Serum Dilution

Figure 10B

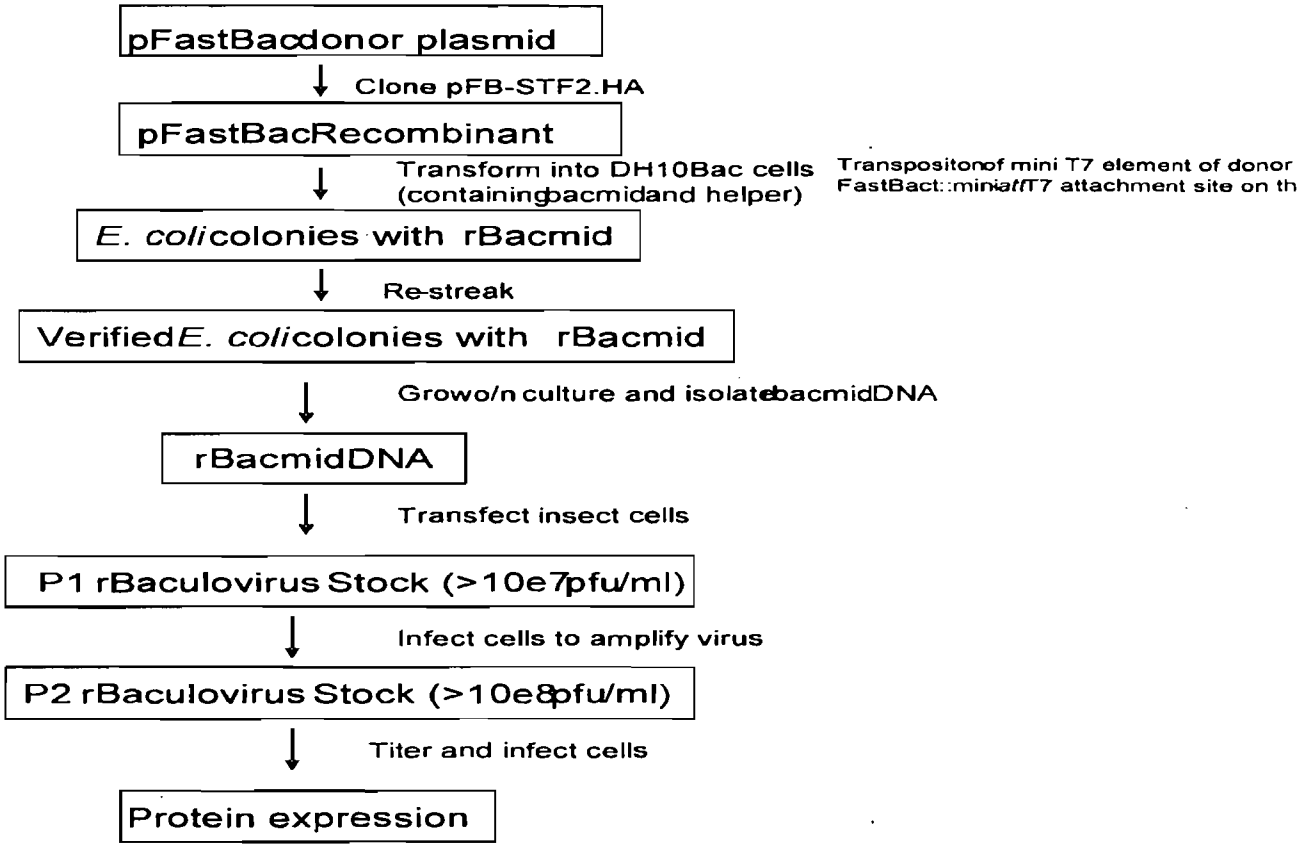

pFastBacdonor plasmid
Clone pFB-STF2.HA
pFastBacRecombinant
Transform into DH10Bac cells (containingbacmidand helper)
Transpositonof mini T7 element of donor FastBact::miniattT7 attachment site on th
E. colicolonies with rBacmid
Re-streak
VerifiedE. colicolonies with rBacmid
Growo/n culture and isolatebacmidDNA
rBacmidDNA
Transfect insect cells
P1 rBaculovirus Stock (>10e7pfu/ml)
Infect cells to amplify virus
P2 rBaculovirus Stock (>10e8pfu/ml)
Titer and infect cells
Protein expression

Figure 11

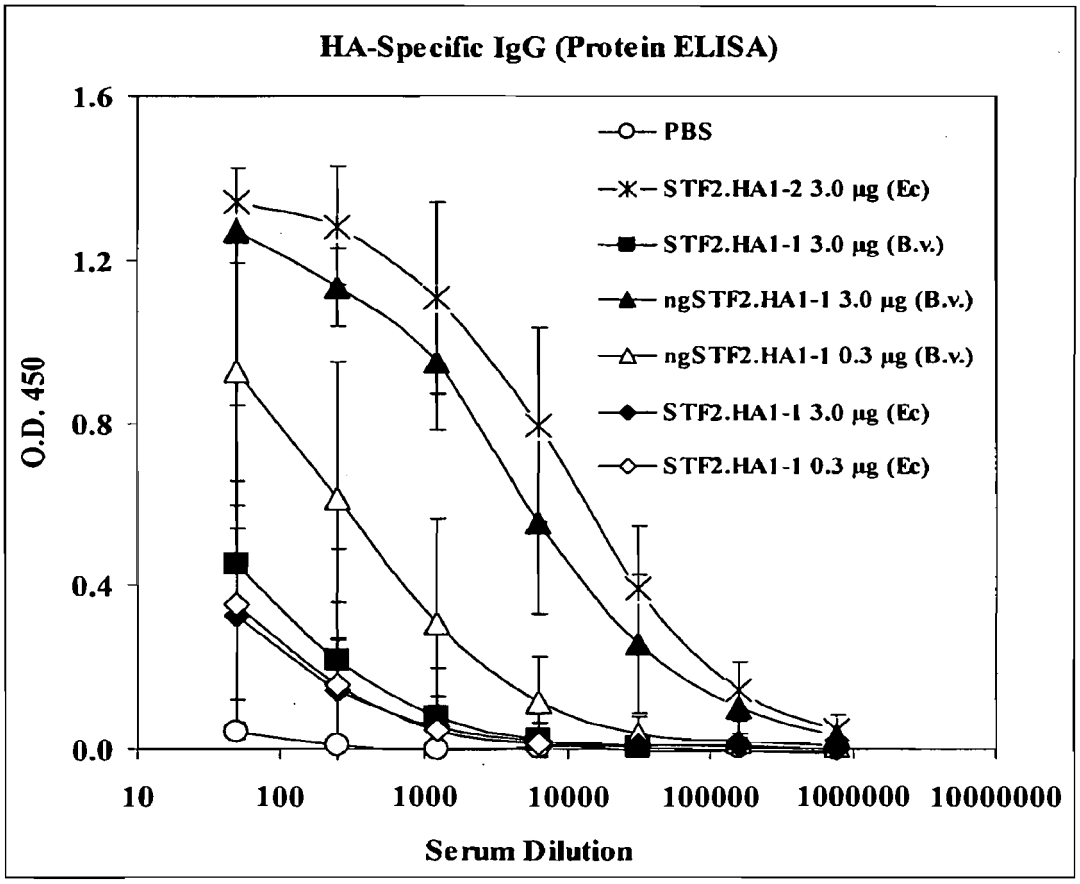

HA-Specific IgG (Protein ELISA)
1.6
1.2
0.8
0.4
0.0
O.D. 450
10
100
1000
10000
100000
1000000
10000000
Serum Dilution
PBS
STF2.HA1-2 3.0 µg (Ec)
STF2.HA1-1 3.0 µg (B.v.)
ngSTF2.HA1-1 3.0 µg (B.v.)
ngSTF2.HA1-1 0.3 µg (B.v.)
STF2.HA1-1 3.0 µg (Ec)
STF2.HA1-1 0.3 µg (Ec)

Figure 12A

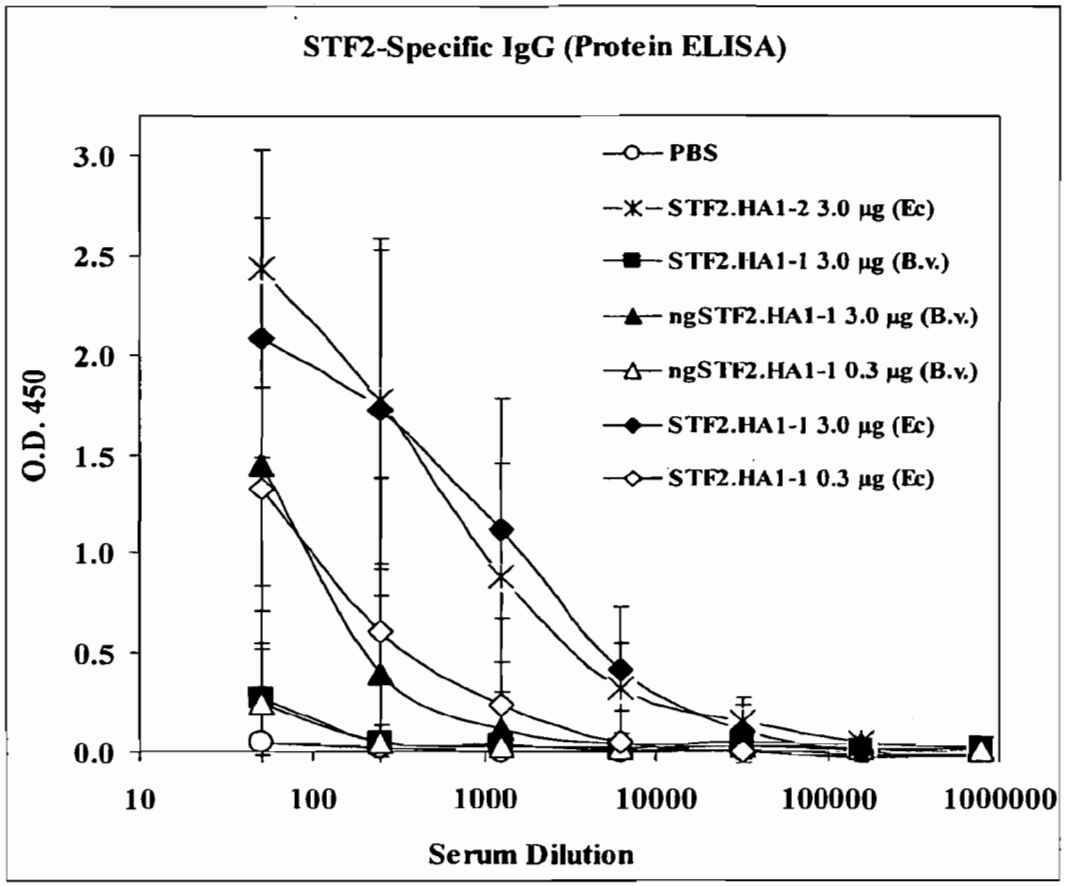

STF2-Specific IgG (Protein ELISA)
O.D. 450
3.0
2.5
2.0
1.5
1.0
0.5
0.0
10
100
1000
10000
100000
1000000
Serum Dilution
PBS
STF2.HA1-2 3.0 µg (Ec)
STF2.HA1-1 3.0 µg (B.v.)
ngSTF2.HA1-1 3.0 µg (B.v.)
ngSTF2.HA1-1 0.3 µg (B.v.)
STF2.HA1-1 3.0 µg (Ec)
STF2.HA1-1 0.3 µg (Ec)

Figure 12B

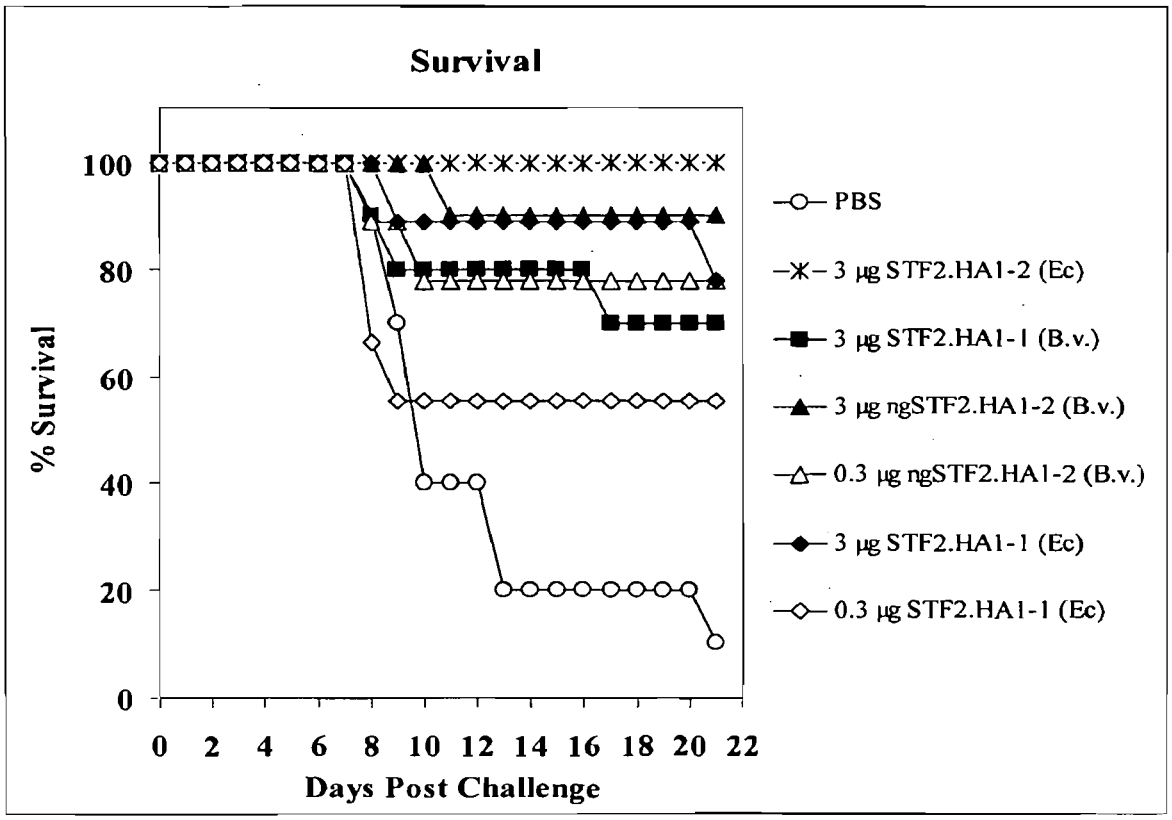

Survival
% Survival
100
80
60
40
20
0
0 2 4 6 8 10 12 14 16 18 20 22
Days Post Challenge
PBS
3 µg STF2.HA1-2 (Ec)
3 µg STF2.HA1-1 (B.v.)
3 µg ngSTF2.HA1-2 (B.v.)
0.3 µg ngSTF2.HA1-2 (B.v.)
3 µg STF2.HA1-1 (Ec)
0.3 µg STF2.HA1-1 (Ec)

Figure 13A

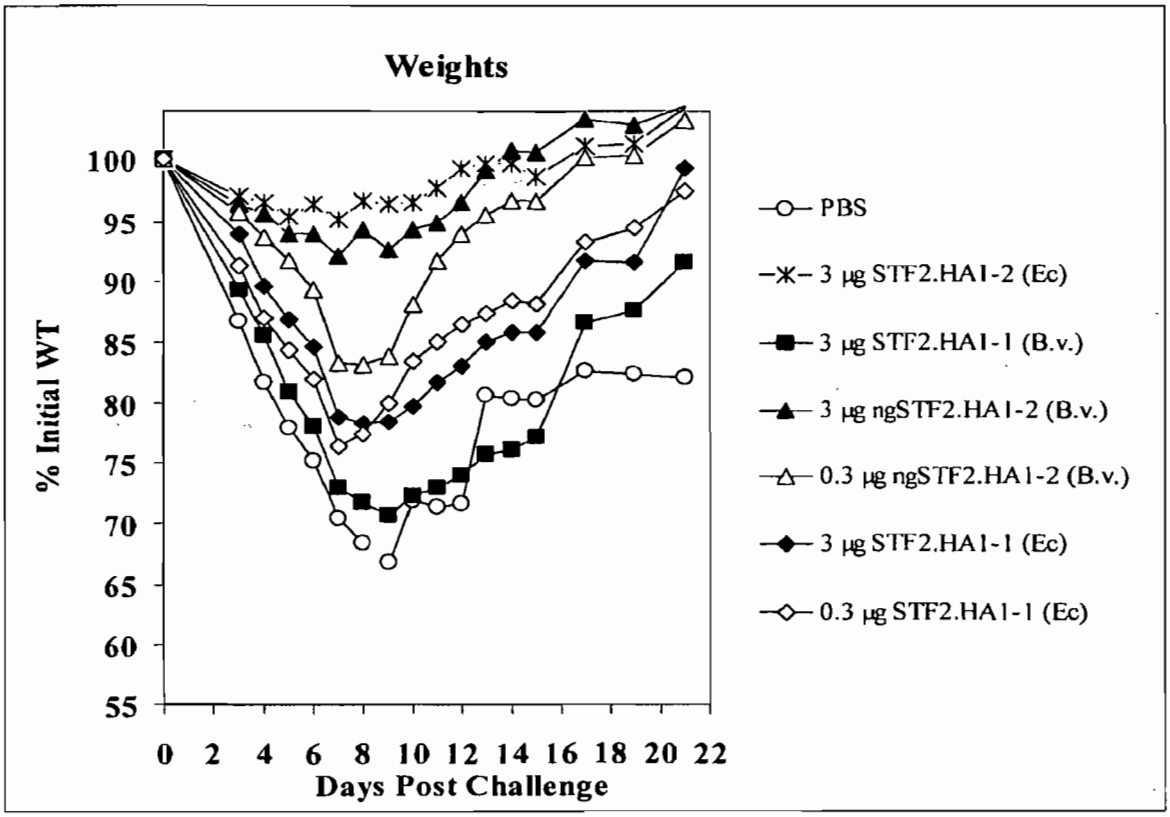

Weights
% Initial WT
100
95
90
85
80
75
70
65
60
55
0 2 4 6 8 10 12 14 16 18 20 22
Days Post Challenge
PBS
3 µg STF2.HA1-2 (Ec)
3 µg STF2.HA1-1 (B.v.)
3 µg ngSTF2.HA1-2 (B.v.)
0.3 µg ngSTF2.HA1-2 (B.v.)
3 µg STF2.HA1-1 (Ec)
0.3 µg STF2.HA1-1 (Ec)

Figure 13B

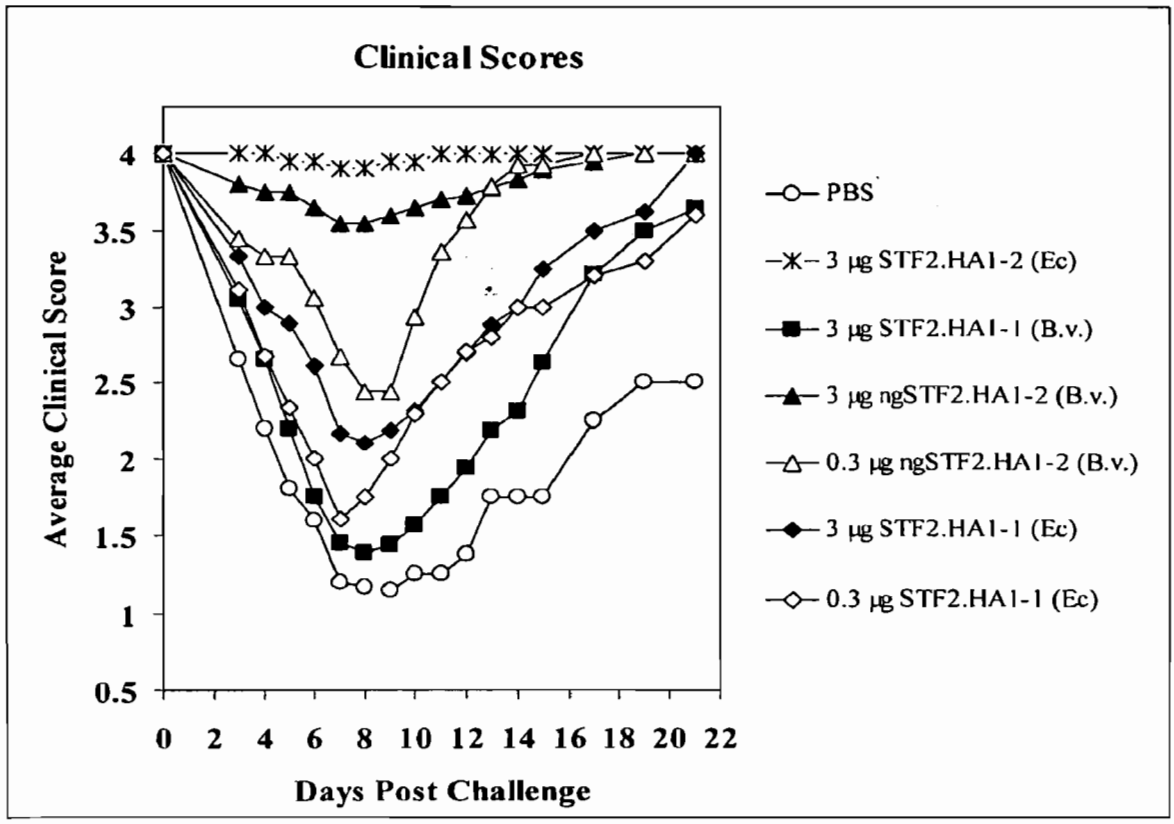

Clinical Scores
Average Clinical Score
4
3.5
3
2.5
2
1.5
1
0.5
0 2 4 6 8 10 12 14 16 18 20 22
Days Post Challenge
PBS
3 µg STF2.HA1-2 (Ec)
3 µg STF2.HA1-1 (B.v.)
3 µg ngSTF2.HA1-2 (B.v.)
0.3 µg ngSTF2.HA1-2 (B.v.)
3 µg STF2.HA1-1 (Ec)
0.3 µg STF2.HA1-1 (Ec)

Figure 13C

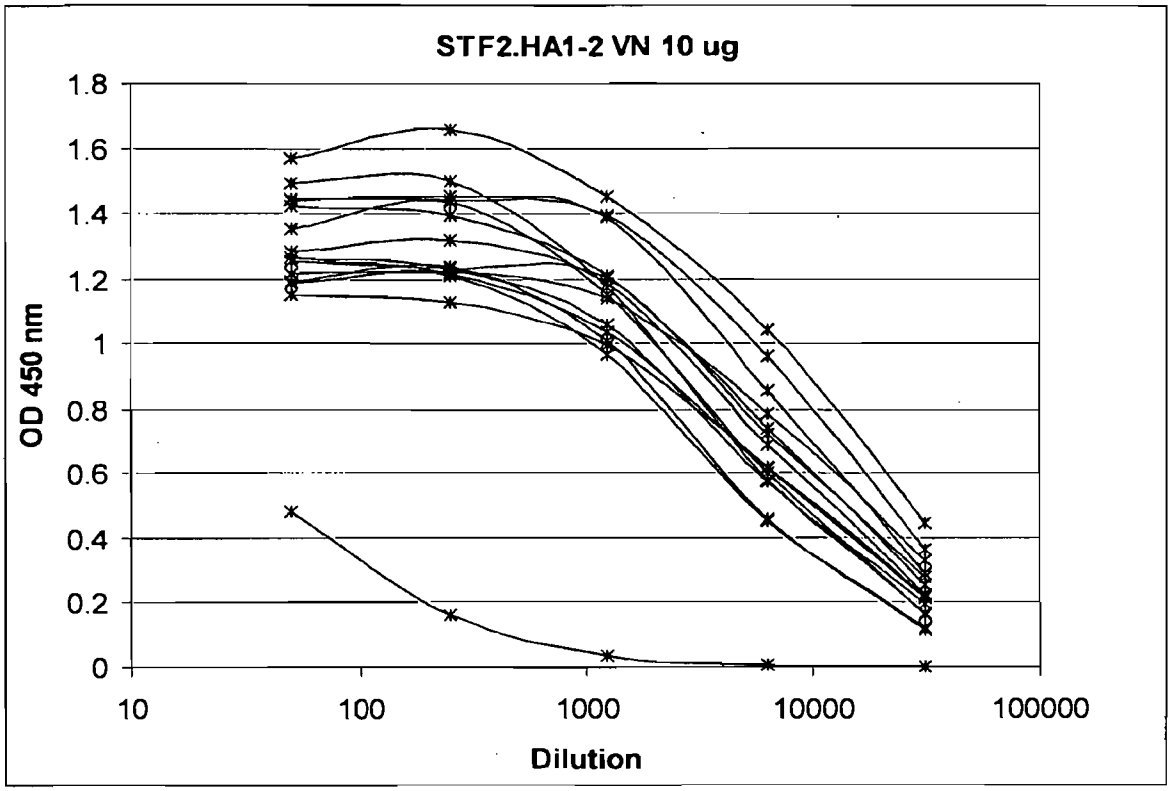

STF2.HA1-2 VN 10 ug
OD 450 nm
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
10
100
1000
10000
100000
Dilution

Figure 14

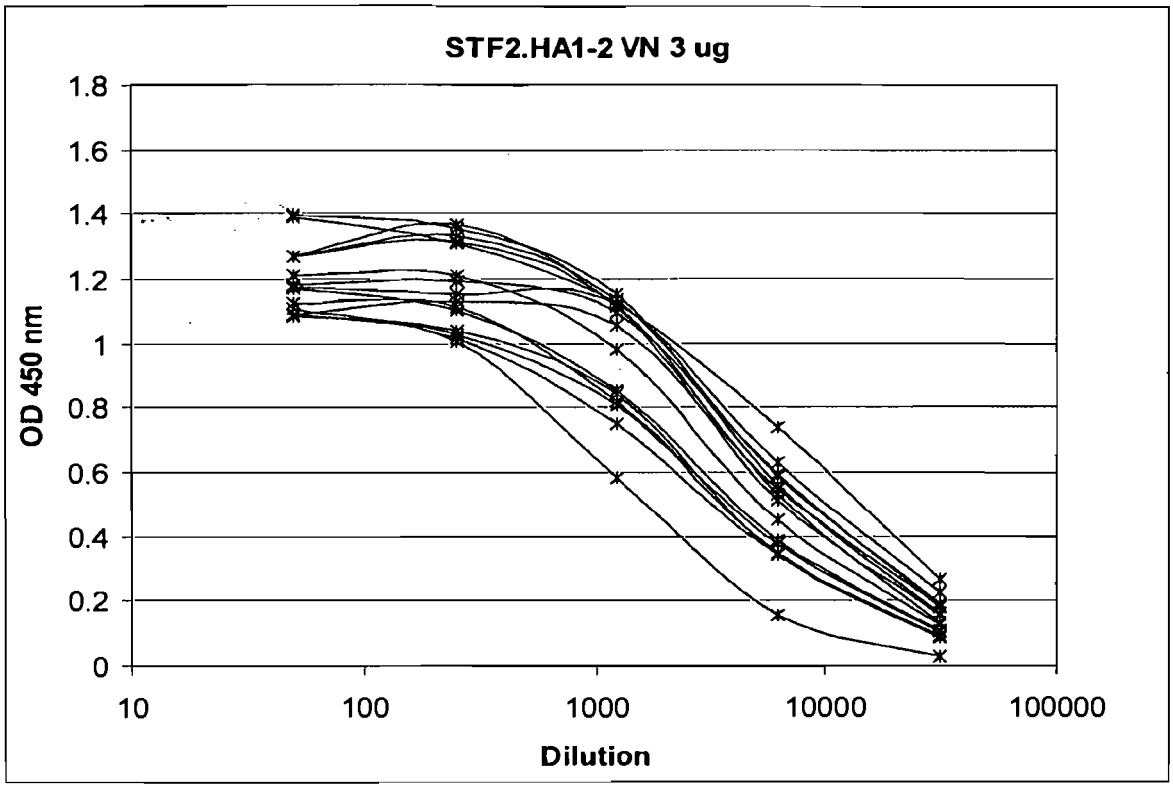

STF2.HA1-2 VN 3 ug
OD 450 nm
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
10
100
1000
10000
100000
Dilution

Figure 15

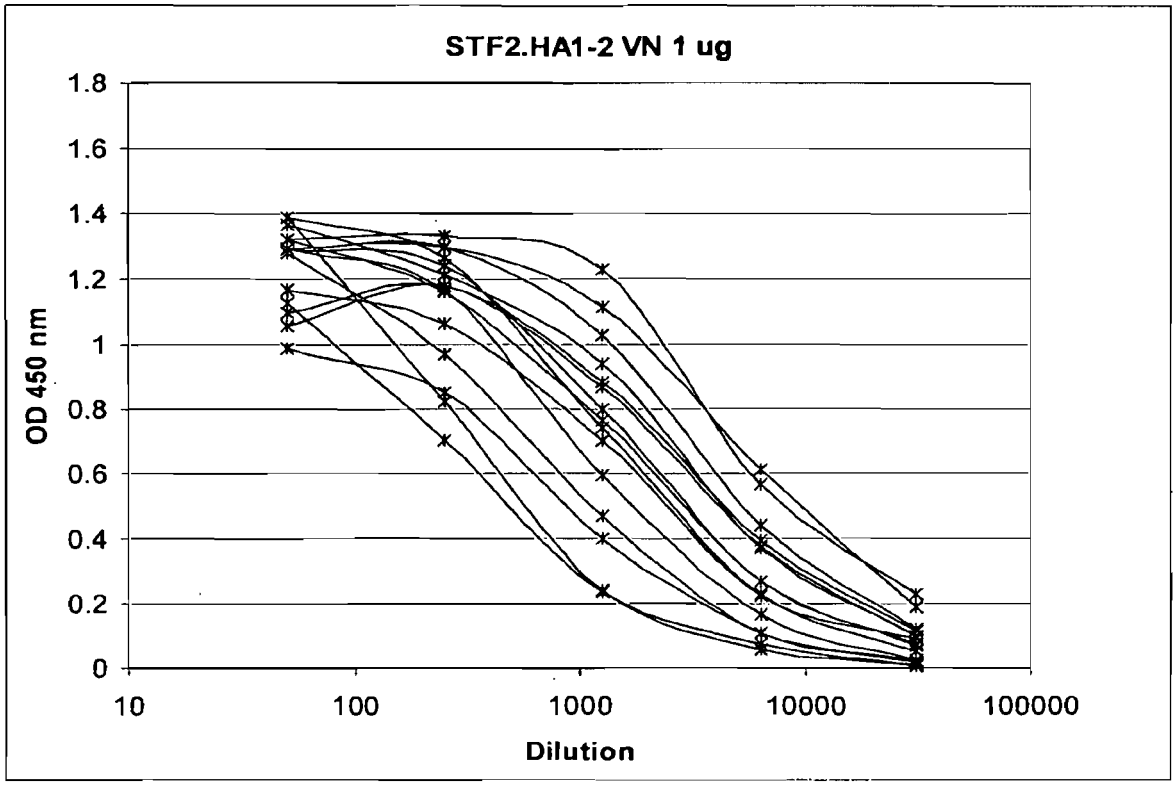

STF2.HA1-2 VN 1 ug
OD 450 nm
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
10
100
1000
10000
100000
Dilution

Figure 16

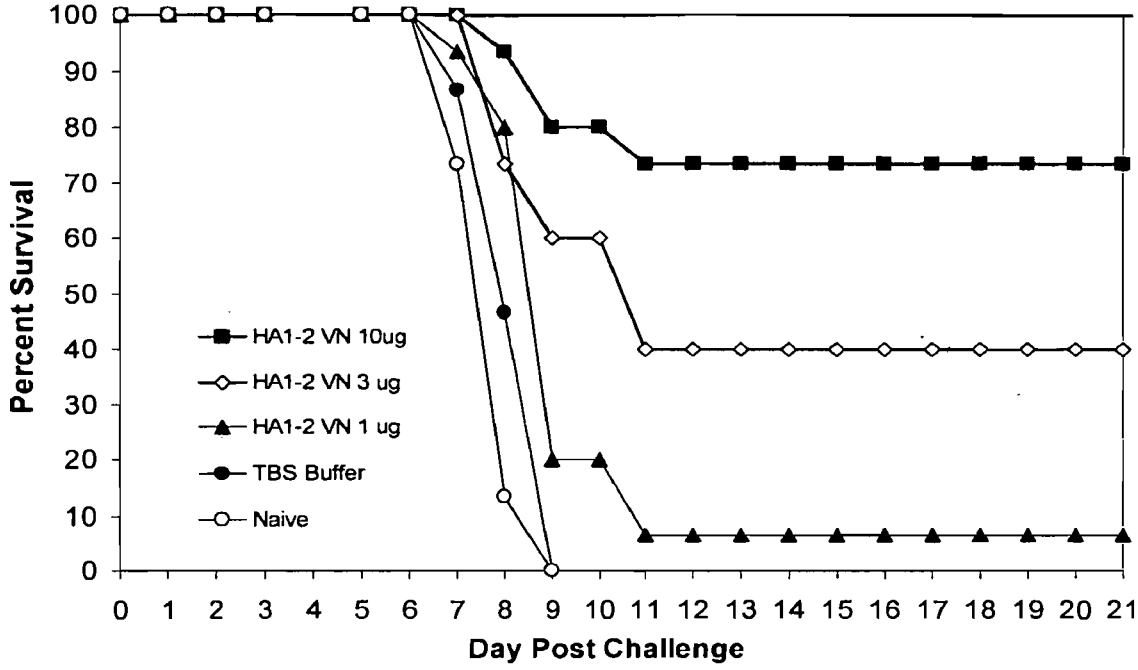

Percent Survival
100
90
80
70
60
50
40
30
20
10
0
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21
Day Post Challenge
HA1-2 VN 10ug
HA1-2 VN 3 ug
HA1-2 VN 1 ug
TBS Buffer
Naive

Figure 17

MAQVINTNSLSLLTQNNLNKSQSALGTAIERLSSGLRINSAKDDAAGQAIANRFTANIKG

LTQASRNANDGISIAQTTEGALNEINNNLQRVRELAVQSANSTNSQSDLDSIQAELTQRL

NEIDRVSGQTQFNGVKVLAQDNTLTIQVGANDGETIDIDLKQINSQTLGLDSLNVQKAYD

VKDTAVTTKAYANNGTTLDVSGLDDAAIKAATGGTNGTASVTGGAVKFDADNNKYFVTIG

GFTGADAAKNGDYEVNVATDGTVTLAAGATKTTMPAGATTKTEVQELKDTPAVVSADAKN

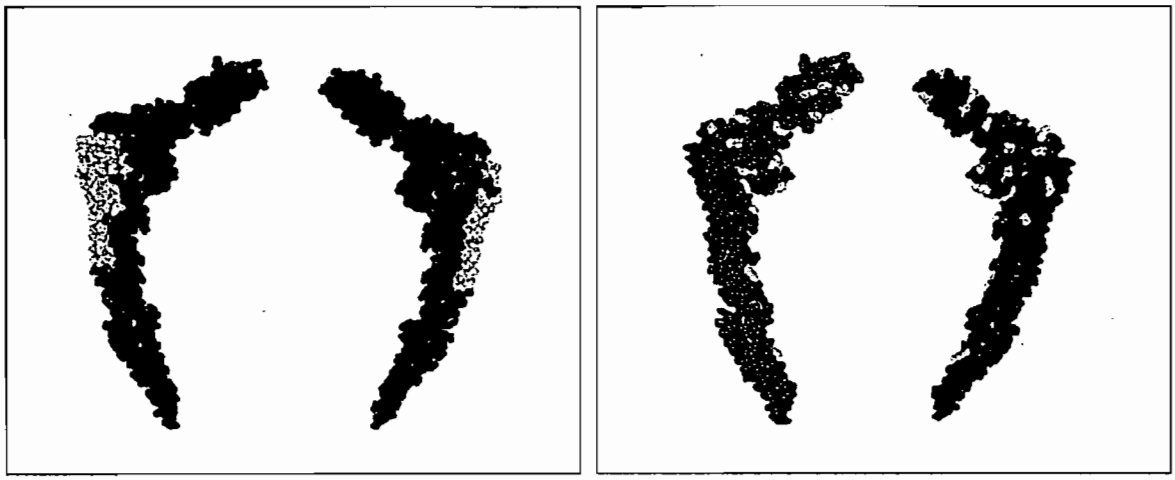
ALIAGGVDATDANGAELVKMSYTDKNGKTIEGGYALKAGDKYYAADYDEATGAIKAKTTS

YTAADGTTKTAANQLGGVDGKTEVVTIDGKTYNASKAAGHDFKAQPELAEAAAKTTENPL

QKIDAALAQVDALRSDLGAVQNRFNSAITNLGNTVNNLSEARSRIEDSDYATEVSNMSRA

QILQQAGTSVLAQANQVPQNVLSLLR

Figure 18



= TLR5 activation = Lysine

Figure 19

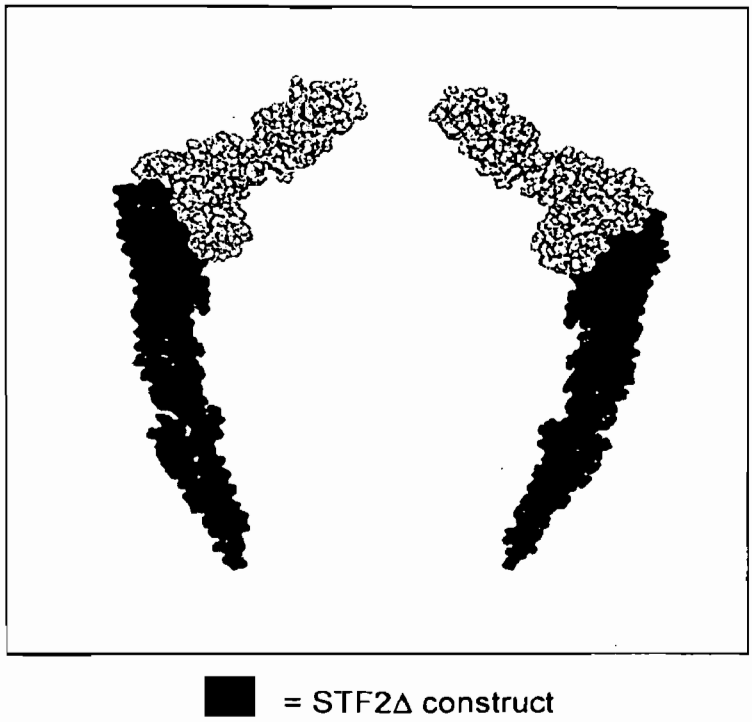


Figure 20

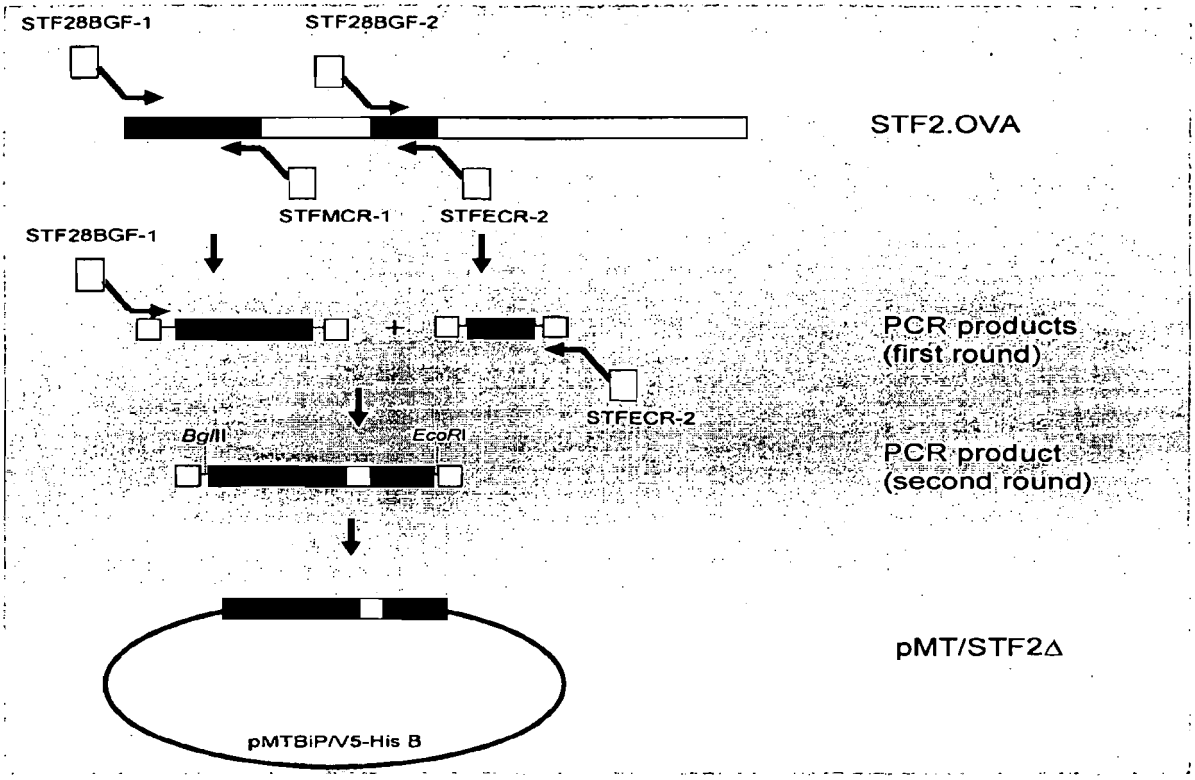

STF28BGF-1
STF28BGF-2
STF2.OVA
STFMCR-1
STFECR-2
STF28BGF-1
PCR products
(first round)
STFECR-2
BglII
EcoRI
PCR product
(second round)
pMT/STF2Δ
pMTBiP/V5-His B

Figure 21

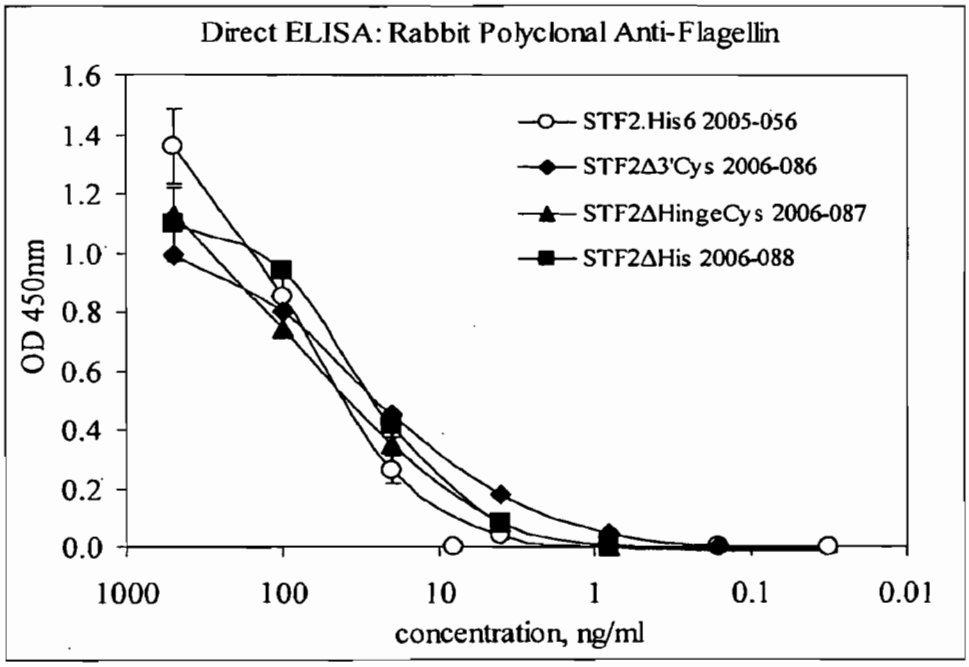

Direct ELISA: Rabbit Polyclonal Anti-Flagellin
OD 450nm
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
1000
100
10
1
0.1
0.01
concentration, ng/ml
STF2.His6 2005-056
STF2Δ3'Cys 2006-086
STF2ΔHingeCys 2006-087
STF2ΔHis 2006-088

Figure 22

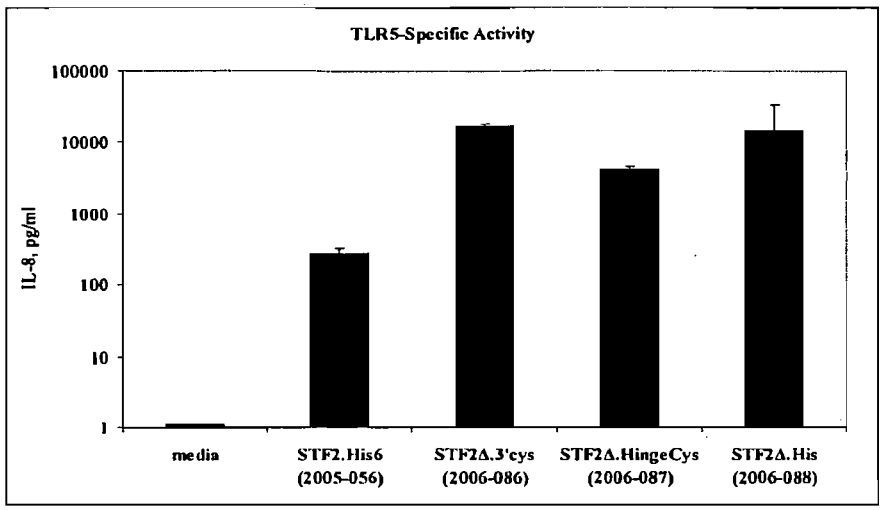

TLR5-Specific Activity
IL-8, pg/ml
100000
10000
1000
100
10
1
media
STF2.His6 (2005-056)
STF2Δ.3'cys (2006-086)
STF2Δ.HingeCys (2006-087)
STF2Δ.His (2006-088)

Figure 23

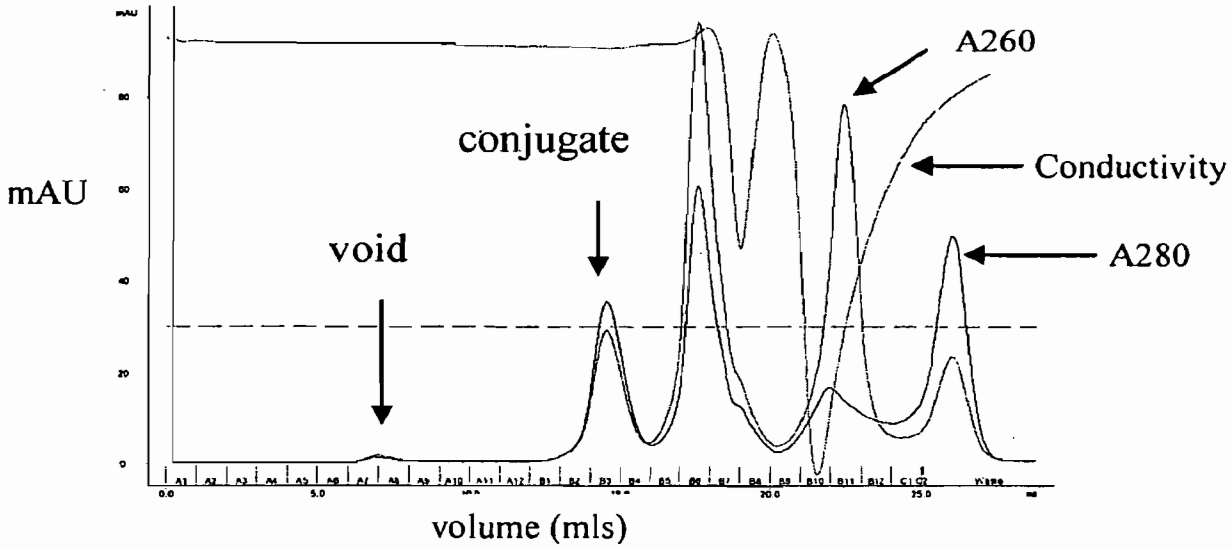


Figure 24

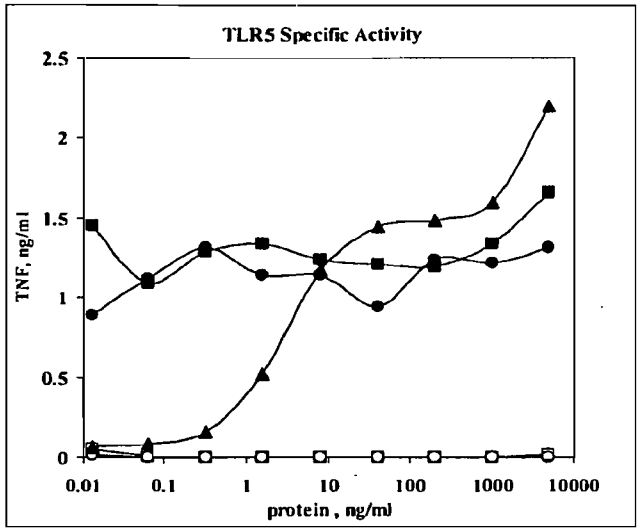

TLR5 Specific Activity
TNF, ng/ml
2.5
2
1.5
1
0.5
0
0.01
0.1
1
10
100
1000
10000
protein , ng/ml

Figure 25

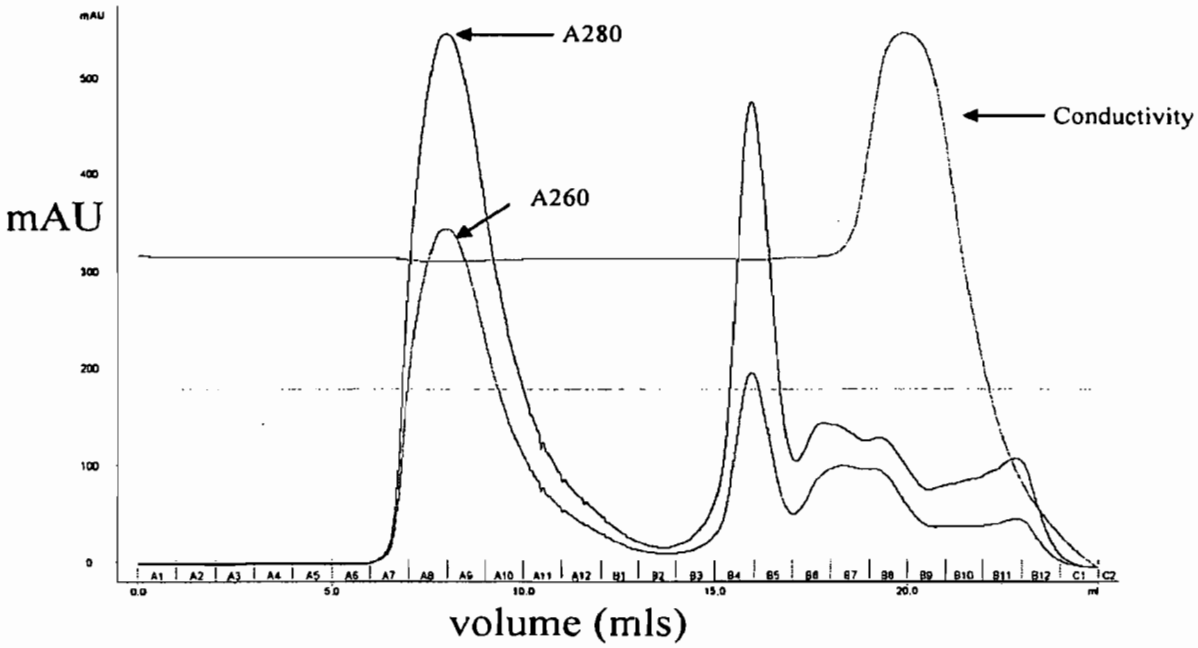

mAU
500
400
300
200
100
0
A280
A260
Conductivity
A1 A2 A3 A4 A5 A6 A7 A8 A9 A10 A11 A12 B1 B2 B3 B4 B5 B6 B7 B8 B9 B10 B11 B12 C1 C2
0.0
5.0
10.0
15.0
20.0
ml
volume (mls)

Figure 26

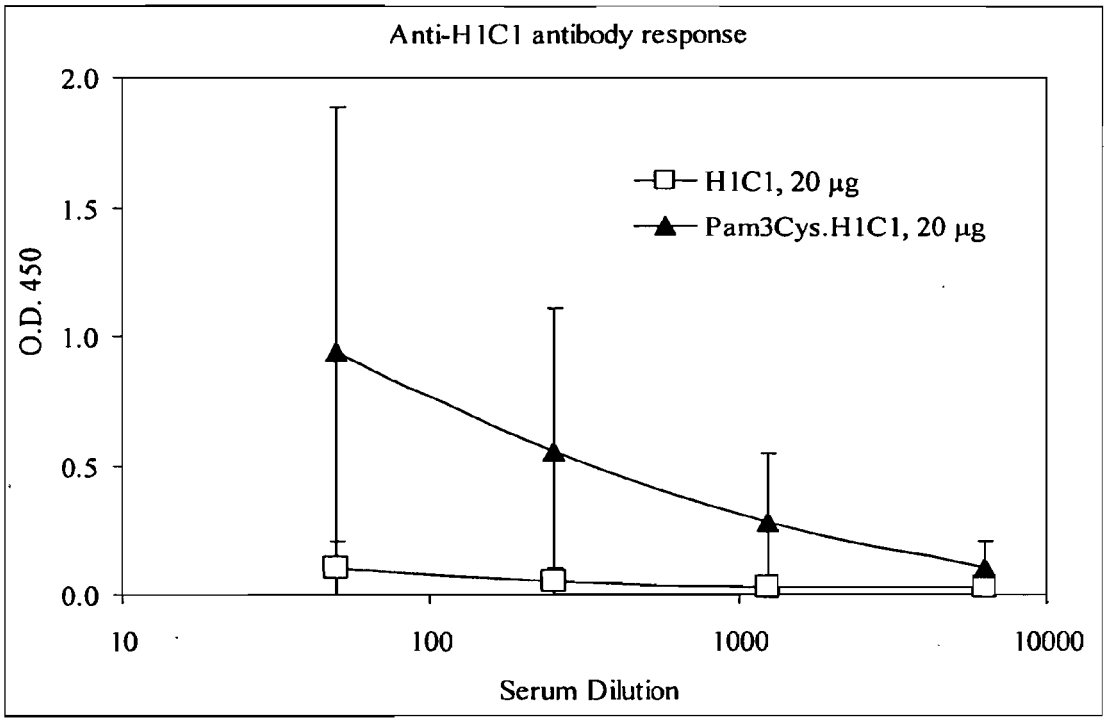

Anti-H1C1 antibody response
2.0
1.5
1.0
0.5
0.0
O.D. 450
H1C1, 20 µg
Pam3Cys.H1C1, 20 µg
10
100
1000
10000
Serum Dilution

Figure 27

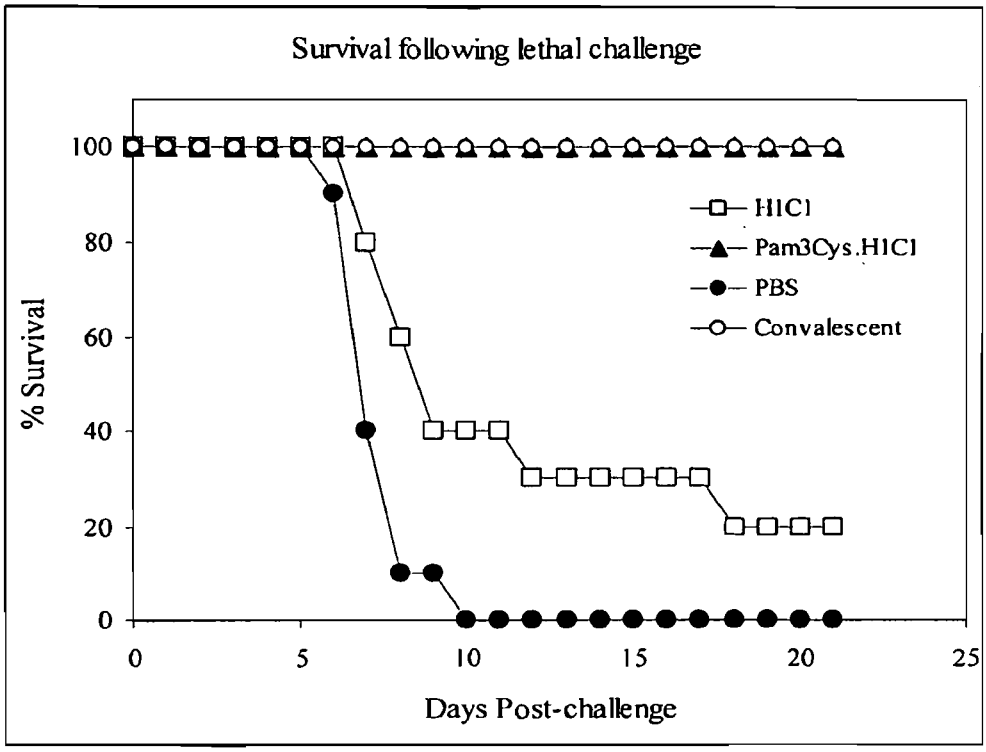

Survival following lethal challenge
100
80
60
40
20
0
% Survival
0
5
10
15
20
25
Days Post-challenge
HICl
Pam3Cys.HICl
PBS
Convalescent

Figure 28

SEQ ID NO: 498 fljB/STF2 amino acid sequence (hinge region underlined)

MAQVINTNSLSLLTQNNLNKSQSALGTAIERLSSGLRINSAKDDAAGQAIANRFTANIKG
LTQASRNANDGISIAQTTEGALNEINNNLQRVRELAVQSANSTNSQSDLDSIQAEITQRL
NEIDRVSGQTQFNGVKVLAQDNTLTIQVGANDGETIDIDLKQINSQTLGLDSLNVQKAYD
VKDTAVTTKAYANNGTTLDVSGLDDAAIKAATGGTNGTASVTGGAVKFDADNNKYFVTIG
GFTGADAAKNGDYEVNVATDGTVTLAAGATKTTMPAGATTKTEVQELKDTPAVVSADAKN
ALIAGGVDATDANGAELVKMSYTDKNGKTIEGGYALKAGDKYYAADYDEATGAIKAKTTS
YTAADGTTKTAANQLGGVDGKTEVVTIDGKTYNASKAAGHDFKAQPELAEAAAKTTENPL
QKIDAALAQVDALRSDLGAVQNRFNSAITNLGNTVNNLSEARSRIEDSDYATEVSNMSRA
QILQQAGTSVLAQANQVPQNVLSLLR

Figure 29

SEQ ID NO: 499 fljB/STF2 nucleic acid sequence (hinge region underlined)

ATGGCACAAGTAATCAACACTAACAGTCTGTCGCTGCTGACCCAGAATAACCTGAACAAA
TCCCAGTCCGCACTGGGCACCGCTATCGAGCGTCTGTCTTCTGGTCTGCGTATCAACAGC
GCGAAAGACGATGCGGCAGGTCAGGCGATTGCTAACCGTTTCACCGCGAACATCAAAGGT
CTGACTCAGGCTTCCCGTAACGCTAACGACGGTATCTCCATTGCGCAGACCACTGAAGGC
GCGCTGAACGAAATCAACAACAACCTGCAGCGTGTGCGTGAACTGGCGGTTCAGTCTGCT
AACAGCACCAACTCCCAGTCTGACCTCGACTCCATCCAGGCTGAAATCACCCAGCGCCTG
AACGAAATCGACCGTGTATCCGGCCAGACTCAGTTCAACGGCGTGAAAGTCCTGGCGCAG
GACAACACCCTGACCATCCAGGTTGGCGCCAACGACGGTGAAACTATCGATATCGATCTG
AAGCAGATCAACTCTCAGACCCTGGGTCTGGACTCACTGAACGTGCAGAAAGCGTATGAT
GTGAAAGATACAGCAGTAACAACGAAAGCTTATGCCAATAATGGTACTACACTGGATGTA
TCGGGTCTTGATGATGCAGCTATTAAAGCGGCTACGGGTGGTACGAATGGTACGGCTTCT
GTAACCGGTGGTGCGGTTAAATTTGACGCAGATAATAACAAGTACTTTGTTACTATTGGT
GGCTTTACTGGTGCTGATGCCGCCAAAAATGGCGATTATGAAGTTAACGTTGCTACTGAC
GGTACAGTAACCCTTGCGGCTGGCGCAACTAAAACCACAATGCCTGCTGGTGCGACAACT
AAAACAGAAGTACAGGAGTTAAAAGATACACCGGCAGTTGTTTCAGCAGATGCTAAAAAT
GCCTTAATTGCTGGCGGCGTTGACGCTACCGATGCTAATGGCGCTGAGTTGGTCAAAATG
TCTTATACCGATAAAAATGGTAAGACAATTGAAGGCGGTTATGCGCTTAAAGCTGGCGAT
AAGTATTACGCCGCAGATTACGATGAAGCGACAGGAGCAATTAAAGCTAAAACTACAAGT
TATACTGCTGCTGACGGCACTACCAAAACAGCGGCTAACCAACTGGGTGGCGTAGACGGT
AAAACCGAAGTCGTTACTATCGACGGTAAAACCTACAATGCCAGCAAAGCCGCTGGTCAT
GATTTCAAAGCACAACCAGAGCTGGCGGAAGCAGCCGCTAAAACCACCGAAAACCCGCTG
CAGAAAATTGATGCCGCGCTGGCGCAGGTGGATGCGCTGCGCTCTGATCTGGGTGCGGTA
CAAAACCGTTTCAACTCTGCTATCACCAACCTGGGCAATACCGTAAACAATCTGTCTGAA
GCGCGTAGCCGTATCGAAGATTCCGACTACGCGACCGAAGTTTCCAACATGTCTCGCGCG
CAGATTCTGCAGCAGGCCGGTACTTCCGTTCTGGCGCAGGCTAACCAGGTCCCGCAGAAC
GTGCTGTCTCTGTTACGT

Figure 30

SEQ ID NO: 500 fljB/STF2Δ amino acid sequence

MAQVINTNSLSLLTQNNLNKSQSALGTAIERLSSGLRINSAKDDAAGQAIANRFTANIKGLT
QASRNANDGISIAQTTEGALNEINNNLQRVRELAVQSANSTNSQSDLDSIQAEITQRLNEID
RVSGQTQFNGVKVLAQDNTLTIQVGANDGETIDIDLKQINSQTLGLDSLNVHGAPVDPASPW
TENPLQKIDAALAQVDALRSDLGAVQNRFNSAITNLGNTVNNLSEARSRIEDSDYATEVSNM
SRAQILQQAGTSVLAQANQVPQNVLSLLR

Figure 31

SEQ ID NO: 501 fljB/STF2Δ nucleic acid sequence

ATGGCACAAGTAATCAACACTAACAGTCTGTCGCTGCTGACCCAGAATAACCTGAACAAATC
CCAGTCCGCACTGGGCACCGCTATCGAGCGTCTGTCTTCTGGTCTGCGTATCAACAGCGCGA
AAGACGATGCGGCAGGTCAGGCGATTGCTAACCGTTTCACCGCGAACATCAAAGGTCTGACT
CAGGCTTCCCGTAACGCTAACGACGGTATCTCCATTGCGCAGACCACTGAAGGCGCGCTGAA
CGAAATCAACAACAACCTGCAGCGTGTGCGTGAACTGGCGGTTCAGTCTGCTAACAGCACCA
ACTCCCAGTCTGACCTCGACTCCATCCAGGCTGAAATCACCCAGCGCCTGAACGAAATCGAC
CGTGTATCCGGCCAGACTCAGTTCAACGGCGTGAAAGTCCTGGCGCAGGACAACACCCTGAC
CATCCAGGTTGGCGCCAACGACGGTGAAACTATCGATATCGATCTGAAGCAGATCAACTCTC
AGACCCTGGGTCTGGACTCACTGAACGTGCATGGAGCGCCGGTGGATCCTGCTAGCCCATGG
ACCGAAAACCCGCTGCAGAAAATTGATGCCGCGCTGGCGCAGGTGGATGCGCTGCGCTCTGA
TCTGGGTGCGGTACAAAACCGTTTCAACTCTGCTATCACCAACCTGGGCAATACCGTAAACA
ATCTGTCTGAAGCGCGTAGCCGTATCGAAGATTCCGACTACGCGACCGAAGTTTCCAACATG
TCTCGCGCGCAGATTTTGCAGCAGGCCGGTACTTCCGTTCTGGCGCAGGCTAACCAGGTCCC
GCAGAACGTGCTGTCTCTGTTACGTG

Figure 32

SEQ ID NO: 502 E. coli fliC amino acid sequence (hinge region underlined)

MAQVINTNSLSLITQNNINKNQSALSSSIERLSSGLRINSAKDDAAGQAIANRFTSNIKG
LTQAARNANDGISVAQTTEGALSEINNNLQRIRELTVQASTGTNSDSDLDSIQDEIKSRL
DEIDRVSGQTQFNGVNVLAKDGSMKIQVGANDGQTITIDLKKIDSDTLGLNGFNVNGSGT
IANKAATISDLTAAKMDAATNTITTTNNALTASKALDQLKDGDTVTIKADAAQTATVYTY
NASAGNFSLSNVSNNTSEKAGDVAASLLPPAGQTASGVYKAASGEVNFDVDANGKITIGG
QKAYLTSDGNLTTNDAGGATAATLDGLFKKAGDGQSIGFKKTASVTMGGTTYNFKTGADA
DAATANAGVSFTDTASKETVLNKVATAKQGKAAAADGDTSATITYKSGVQTYQAVFAAGD
GTASAKYADKADVSNATATYTDADGEMTTIGSYTTKYSIDANNGKVTVDSGTGTGKYAPK
VGAEVYVSANGTLTTDATSEGTVTKDPLKALDEAISSIDKFRSSLGAIQNRLDSAVTNLN
NTTTNLSEAQSRIQDADYATEVSNMSKAQIIQQAGNSVLAKANQVPQQVLSLLQG

Figure 33

SEQ ID NO: 503 E. coli fliC -nucleic acid sequence (hinge region underlined)

ATGGCACAAGTCATTAATACCAACAGCCTCTCGCTGATCACTCAAAATAATATCAACAAG
AACCAGTCTGCGCTGTCGAGTTCTATCGAGCGTCTGTCTTCTGGCTTGCGTATTAACAGC
GCGAAGGATGACGCCGCAGGTCAGGCGATTGCTAACCGTTTTACTTCTAACATTAAAGGC
CTGACTCAGGCTGCACGTAACGCCAACGACGGTATTTCCGTTGCGCAGACCACCGAAGGC
GCGCTGTCCGAAATCAACAACAACTTACAGCGTATCCGTGAACTGACGGTTCAGGCTTCT
ACCGGGACTAACTCCGATTCAGATCTGGACTCCATTCAGGACGAAATCAAATCCCGTCTG
GACGAAATTGACCGCGTATCTGGCCAGACCCAGTTCAACGGCGTGAACGTACTGGCGAAA
GACGGTTCAATGAAAATTCAGGTTGGTGCGAATGACGGCCAGACTATCACGATTGATCTG
AAGAAAATTGACTCAGATACGCTGGGGCTGAATGGTTTTAACGTGAATGGTTCCGGTACG
ATAGCCAATAAAGCGGCGACCATTAGCGACCTGACAGCAGCGAAAATGGATGCTGCAACT
AATACTATAACTACAACAAATAATGCGCTGACTGCATCAAAGGCGCTTGATCAACTGAAA
GATGGTGACACTGTTACTATCAAAGCAGATGCTGCTCAAACTGCCACGGTTTATACATAC
AATGCATCAGCTGGTAACTTCTCACTCAGTAATGTATCGAATAATACTTCAGAAAAAGCA
GGTGATGTAGCAGCTAGCCTTCTCCCGCCGGCTGGGCAAACTGCTAGTGGTGTTTATAAA
GCAGCAAGCGGTGAAGTGAACTTTGATGTTGATGCGAATGGTAAAATCACAATCGGAGGA
CAGAAAGCATATTTAACTAGTGATGGTAACTTAACTACAAACGATGCTGGTGGTGCGACT
GCGGCTACGCTTGATGGTTTATTCAAGAAAGCTGGTGATGGTCAATCAATCGGGTTTAAG
AAGACTGCATCAGTCACGATGGGGGGAACAACTTATAACTTTAAAACGGGTGCTGATGCT
GATGCTGCAACTGCTAACGCAGGGGTATCGTTCACTGATACAGCTAGCAAAGAAACCGTT
TTAAATAAAGTGGCTACAGCTAAACAAGGCAAAGCAGCTGCAGCTGACGGTGATACATCC
GCAACAATTACCTATAAATCTGGCGTTCAGACGTATCAGGCTGTATTTGCCGCAGGTGAC
GGTACTGCTAGCGCAAAATATGCCGATAAAGCTGACGTTTCTAATGCAACAGCAACATAC
ACTGATGCTGATGGTGAAATGACTACAATTGGTTCATACACCACGAAGTATTCAATCGAT
GCTAACAACGGCAAGGTAACTGTTGATTCTGGAACTGGTACGGGTAAATATGCGCCGAAA
GTAGGGGCTGAAGTATATGTTAGTGCTAATGGTACTTTAACAACAGATGCAACTAGCGAA
GGCACAGTAACAAAAGATCCACTGAAAGCTCTGGATGAAGCTATCAGCTCCATCGACAAA
TTCCGTTCTTCCCTGGGTGCTATCCAGAACCGTCTGGATTCCGCAGTCACCAACCTGAAC
AACACCACTACCAACCTGTCCGAAGCGCAGTCCCGTATTCAGGACGCCGACTATGCGACC
GAAGTGTCCAACATGTCGAAAGCGCAGATCATTCAGCAGGCCGGTAACTCCGTGCTGGCA
AAAGCCAACCAGGTACCGCAGCAGGTTCTGTCTCTGCTGCAGGGTTAG

Figure 34

SEQ ID NO: 504 Salmonella muenchen flagellin fliC amino acid sequence (hinge region underlined)

MAQVINTNSLSLLTQNNLNKSQSALGTAIERLSSGLRINSAKDDAAGQAIANRFTANIKGLT
QASRNANDGISIAQTTEGALNEINNNLQRVRELAVQSANGTNSQSDLDSIQAEITQRLNEID
RVSGQTQFNGVKVLAQDNTLTIQVGANDGETIDIDLKEISSKTLGLDKLNVQDAYTPKETAV
TVDKTTYKNGTDTITAQSNTDIQTAIGGGATGVTGADIKFKDGQYYLDVKGGASAGVYKATY
DETTKKVNIDTTDKTPLATAEATAIRGTATITHNQIAEVTKEGVDTTTVAAQLAAAGVTGAD
KDNTSLVKLSFEDKNGKVIDGGYAVKMGDDFYAATYDEKTGTITAKTTTYTDGAGVAQTGAV
KFGGANGKSEVVTATDGKTYLASDLDKHNFRTGGELKEVNTDKTENPLQKIDAALAQVDTLR
SDLGAVQNRFNSAITNLGNTVNNLSSARSRIEDSDYATEVSNMSRAQILQQAGTSVLAQANQ
VPQNVLSLLR

Figure 35

SEQ ID NO: 505 Salmonella muenchen flagellin fliC nucleic acid sequence (hinge region underlined)

AATGGCACAAGTCATTAATACAAACAGCCTGTCGCTGTTGACCCAGAATAACCTGAACAAAT
CCCAGTCCGCTCTGGGCACCGCTATCGAGCGTCTGTCTTCCGGTCTGCGTATCAACAGCGCG
AAAGACGATGCGGCAGGTCAGGCGATTGCTAACCGTTTCACCGCGAACATCAAAGGTCTGAC
TCAGGCTTCCCGTAACGCTAACGACGGTATCTCCATTGCGCAGACCACTGAAGGCGCGCTGA
ACGAAATCAACAACAACCTGCAGCGTGTGCGTGAACTGGCGGTTCAGTCTGCTAACGGTACT
AACTCCCAGTCTGACCTTGACTCTATCCAGGCTGAAATCACCCAGCGTCTGAACGAAATCGA
CCGTGTATCCGGTCAGACTCAGTTCAACGGCGTGAAAGTCCTGGCGCAGGACAACACCCTGA
CCATCCAGGTTGGTGCCAACGACGGTGAAACTATTGATATTGATTTAAAAGAAATTAGCTCT
AAAACACTGGGACTTGATAAGCTTAATGTCCAGGATGCCTACACCCCGAAAGAAACTGCTGT
AACCGTTGATAAAACTACCTATAAAAATGGTACAGATACTATTACAGCCCAGAGCAATACTG
ATATCCAAACTGCAATTGGCGGTGGTGCAACGGGGGTTACTGGGGCTGATATCAAATTTAAA
GATGGTCAATACTATTTAGATGTTAAAGGCGGTGCTTCTGCTGGTGTTTATAAAGCCACTTA
TGATGAAACTACAAAGAAAGTTAATATTGATACGACTGATAAAACTCCGTTAGCAACTGCGG
AAGCTACAGCTATTCGGGGAACGGCCACTATAACCCACAACCAAATTGCTGAAGTAACAAAA
GAGGGTGTTGATACGACCACAGTTGCGGCTCAACTTGCTGCTGCAGGGGTTACTGGTGCCGA
TAAGGACAATACTAGCCTTGTAAAACTATCGTTTGAGGATAAAAACGGTAAGGTTATTGATG
GTGGCTATGCAGTGAAAATGGGCGACGATTTCTATGCCGCTACATATGATGAGAAAACAGGT
ACAATTACTGCTAAAACAACCACTTATACAGATGGTGCTGGCGTTGCTCAAACTGGAGCTGT
GAAATTTGGTGGCGCAAATGGTAAATCTGAAGTTGTTACTGCTACCGATGGTAAAACTTACT
TAGCAAGCGACCTTGACAAACATAACTTCAGAACAGGCGGTGAGCTTAAAGAGGTTAATACA
GATAAGACTGAAAACCCACTGCAGAAAATTGATGCTGCCTTGGCACAGGTTGATACACTTCG
TTCTGACCTGGGTGCGGTACAGAACCGTTTCAACTCCGCTATCACCAACCTGGGCAATACCG
TAAATAACCTGTCTTCTGCCCGTAGCCGTATCGAAGATTCCGACTACGCGACCGAAGTCTCC
AACATGTCTCGCGCGCAGATTCTGCAGCAGGCCGGTACCTCCGTTCTGGCGCAGGCTAACCA
GGTTCCGCAAAACGTCCTCTCTTTACTGCGTTAA

Figure 36

SEQ ID:506 Amino acid sequence of pMT/STF2 (Linker underlined)

MKLCILLAVVAFVGLSLGRSAQVINTNSLSLLTQNNLNKSQSALGTAIERLSSGLRI
NSAKDDAAGQAIANRFTANIKGLTQASRNANDGISIAQTTEGALNEINNNLQRVREL
AVQSANSTNSQSDLDSIQAEITQRLNEIDRVSGQTQFNGVKVLAQDNTLTIQVGAND
GETIDIDLKQINSQTLGLDSLNVQKAYDVKDTAVTTKAYANNGTTLDVSGLDDAAIK
AATGGTNGTASVTGGAVKFDADNNKYFVTIGGFTGADAAKNGDYEVNVATDGTVTLA
AGATKTTMPAGATTKTEVQELKDTPAVVSADAKNALIAGGVDATDANGAELVKMSYT
DKNGKTIEGGYALKAGDKYYAADYDEATGAIKAKTTSYTAADGTTKTAANQLGGVDG
KTEVVTIDGKTYNASKAAGHDFKAQPELAEAAAKTTENPLQKIDAALAQVDALRSDL
GAVQNRFNSAITNLGNTVNNLSEARSRIEDSDYATEVSNMSRAQILQQAGTSVLAQA
NQVPQNVLSLLRKGNSKLEGQLEFPRTSPVWWNSADIQHSGGRSSLEGPRFEGKPIP
NPLLGLDSTRTGHHHHHH

Figure 37

SEQ ID: 507 Amino acid sequence of pMT/STF2 (Linker underlined)

ATGAAGTTATGCATATTACTGGCCGTCGTGGCCTTTGTTGGCCTCTCGCTCGGGAGATCT
GCACAAGTAATCAACACTAACAGTCTGTCGCTGCTGACCCAGAATAACCTGAACAAATCC
CAGTCCGCACTGGGCACCGCTATCGAGCGTCTGTCTTCTGGTCTGCGTATCAACAGCGCG
AAAGACGATGCGGCAGGTCAGGCGATTGCTAACCGTTTCACCGCGAACATCAAAGGTCTG
ACTCAGGCTTCCCGTAACGCTAACGACGGTATCTCCATTGCGCAGACCACTGAAGGCGCG
CTGAACGAAATCAACAACAACCTGCAGCGTGTGCGTGAACTGGCGGTTCAGTCTGCTAAC
AGCACCAACTCCCAGTCTGACCTCGACTCCATCCAGGCTGAAATCACCCAGCGCCTGAAC
GAAATCGACCGTGTATCCGGCCAGACTCAGTTCAACGGCGTGAAAGTCCTGGCGCAGGAC
AACACCCTGACCATCCAGGTTGGCGCCAACGACGGTGAAACTATCGATATCGATCTGAAG
CAGATCAACTCTCAGACCCTGGGTCTGGACTCACTGAACGTGCAGAAAGCGTATGATGTG
AAAGATACAGCAGTAACAACGAAAGCTTATGCCAATAATGGTACTACACTGGATGTATCG
GGTCTTGATGATGCAGCTATTAAAGCGGCTACGGGTGGTACGAATGGTACGGCTTCTGTA
ACCGGTGGTGCGGTTAAATTTGACGCAGATAATAACAAGTACTTTGTTACTATTGGTGGC
TTTACTGGTGCTGATGCCGCCAAAAATGGCGATTATGAAGTTAACGTTGCTACTGACGGT
ACAGTAACCCTTGCGGCTGGCGCAACTAAAACCACAATGCCTGCTGGTGCGACAACTAAA
ACAGAAGTACAGGAGTTAAAAGATACACCGGCAGTTGTTTCAGCAGATGCTAAAAATGCC
TTAATTGCTGGCGGCGTTGACGCTACCGATGCTAATGGCGCTGAGTTGGTCAAAATGTCT
TATACCGATAAAAATGGTAAGACAATTGAAGGCGGTTATGCGCTTAAAGCTGGCGATAAG
TATTACGCCGCAGATTACGATGAAGCGACAGGAGCAATTAAAGCTAAAACTACAAGTTAT
ACTGCTGCTGACGGCACTACCAAAACAGCGGCTAACCAACTGGGTGGCGTAGACGGTAAA
ACCGAAGTCGTTACTATCGACGGTAAAACCTACAATGCCAGCAAAGCCGCTGGTCATGAT
TTCAAAGCACAACCAGAGCTGGCGGAAGCAGCCGCTAAAACCACCGAAAACCCGCTGCAG
AAAATTGATGCCGCGCTGGCGCAGGTGGATGCGCTGCGCTCTGATCTGGGTGCGGTACAA
AACCGTTTCAACTCTGCTATCACCAACCTGGGCAATACCGTAAACAATCTGTCTGAAGCG
CGTAGCCGTATCGAAGATTCCGACTACGCGACCGAAGTTTCCAACATGTCTCGCGCGCAG
ATTCTGCAGCAGGCCGGTACTTCCGTTCTGGCGCAGGCTAACCAGGTCCCGCAGAACGTG
CTGTCTCTGTTACGTAAGGGCAATTCGAAGCTTGAAGGTCAATTGGAATTCCCTAGGACT
AGTCCAGTGTGGTGGAATTCTGCAGATATCCAGCACAGTGGCGGCCGCTCGAGTCTAGAG
GGCCCGCGGTTCGAAGGTAAGCCTATCCCTAACCCTCTCCTCGGTCTCGATTCTACGCGT
ACCGGTCATCATCACCATCACCAT

Figure 38



Figure 39

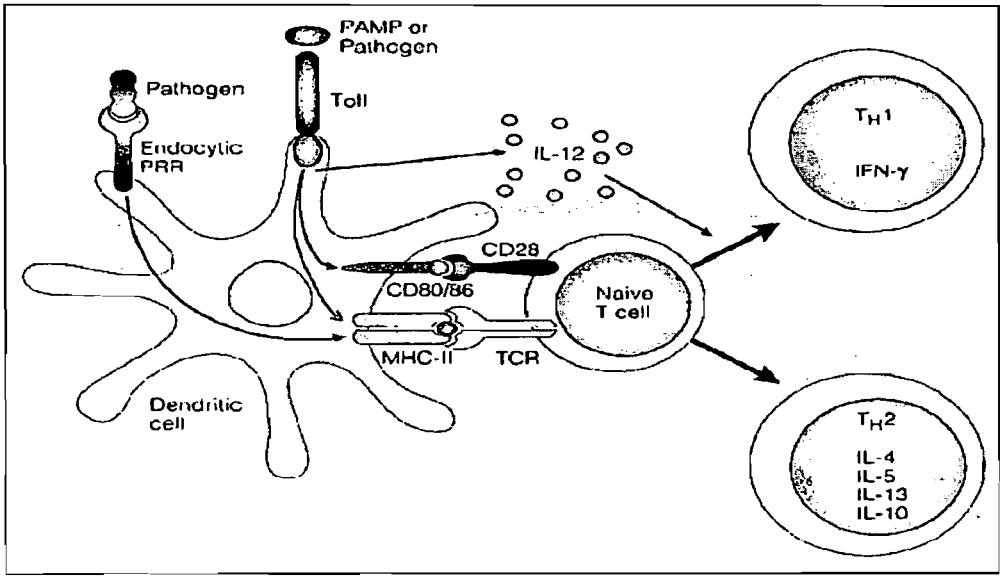

PAMP or
Pathogen
Pathogen
Toll
Endocytic
PRR
IL-12
T_H1
IFN-γ
CD28
CD80/86
Naive
T cell
MHC-II
TCR
Dendritic
cell
T_H2
IL-4
IL-5
IL-13
IL-10

Figure 40

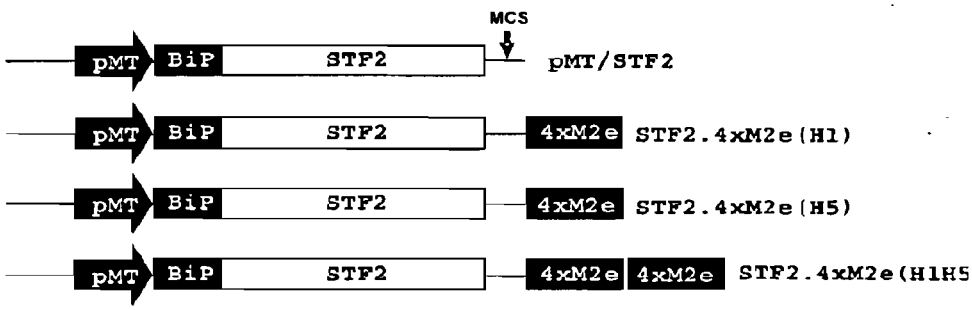

MCS
pMT
BiP
STF2
pMT/STF2
pMT
BiP
STF2
4xM2e
STF2.4xM2e(H1)
pMT
BiP
STF2
4xM2e
STF2.4xM2e(H5)
pMT
BiP
STF2
4xM2e
4xM2e
STF2.4xM2e(H1H5

Figure 41A

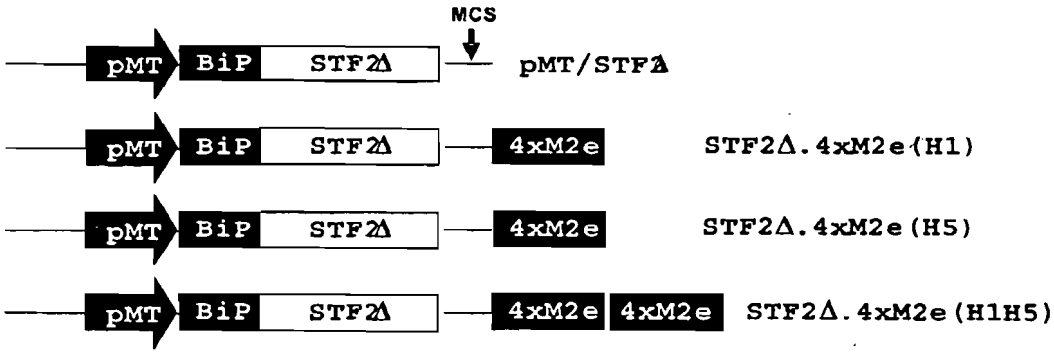

MCS
pMT
BiP
STF2Δ
pMT/STF2Δ
4xM2e
STF2Δ.4xM2e(H1)
STF2Δ.4xM2e(H5)
4xM2e
STF2Δ.4xM2e(H1H5)

Figure 41B

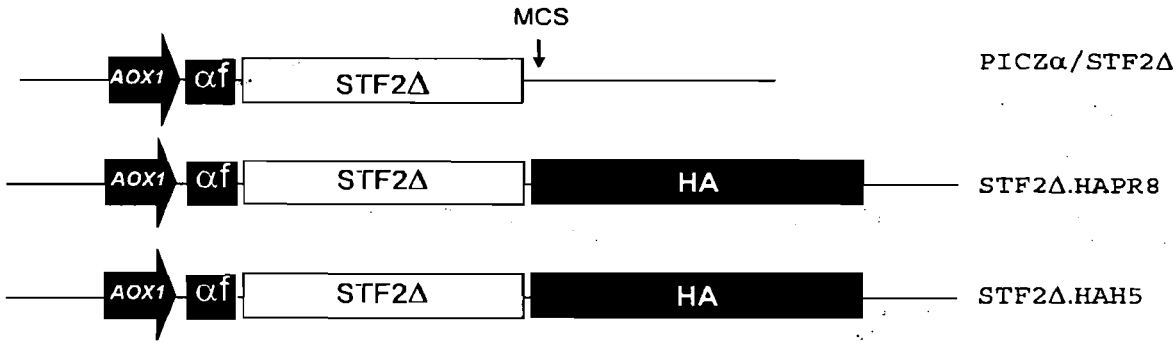

MCS
AOX1
αf
STF2Δ
PICZα/STF2Δ
AOX1
αf
STF2Δ
HA
STF2Δ.HAPR8
AOX1
αf
STF2Δ
HA
STF2Δ.HAH5

Figure 42

SEQ ID NO: 561

DTICIGYHANNSTDTVDTVLEKNVTVTHSVNLLEDSHNGKLCRLKGIAPLQLGKCNIAGW
LLGNPECDPLLPVRSWSYIVETPNSENGICYPGDFIDYEELREQLSSVSSFERFEIFPKE
SSWPNHNTNGVTAACSHEGKSSFYRNLLWLTEKEGSYPKLKNSYVNKKGKEVLVLWGIHH
PPNSKEQQNLYQNENAYVSVVTSNYNRRFTPEIAERPKVRDQAGRMNYYWTLLKPGDTII
FEANGNLIAPMYAFALSRGFGSGIITSNASMHECNTKCQTPLGAINSSLPYQNIHPVTIG
ECPKYVRSAKLRMVTGLRNIPSIQSRGLFGAIAGFIEGGWTGMIDGWYGYHHQNEQGSGY
AADQKSTQNAINGITNKVNTVIEKMNIQFTAVGKEFNKLEKRMENLNKKVDDGFLDIWTY
NAELLVLLENERTLDFHDSNVKNLYEKVKSQLKNNAKEIGNGCFEFYHKCDNECMESVRN
GTYDYPKYSEESKLNREKVDGVKLESMGIYQ

Figure 43

SEQ ID NO: 562

GACCAGATCTGTATCGGTTATCATGCTAACAATTCTACTGAACAAGTAGATACTATCATG
GAGAAGAACGTTACAGTTACACATGCACAAGATATCCTGGAAAAGAAGCATAATGGAAAA
CTGTGTGACCTTGATGGTGTTAAACCACTAATATTGCGTGACTGCTCAGTTGCTGGGTGG
TTGTTGGGGAATCCAATGTGCGACGAATTTATCAACGTTCCAGAATGGAGTTACATTGTT
GAAAAAGCTAACCCTGTTAACGACTTGTGTTACCCAGGCGATTTTAATGACTACGAGGAA
CTTAAGCATTTGTTGTCAAGAATTAACCACTTCGAGAAAATTCAAATTATTCCAAAGTCA
TCTTGGTCCTCCCATGAAGCATCCCTAGGAGTCTCTTCCGCTTGCCCTTACCAAGGCAAG
AGTTCCTTTTTTCGTAATGTCGTCTGGCTGATCAAAAAGAACTCCACCTATCCAACTATA
AAGAGATCATACAACAACACAAATCAGGAGGATCTGCTAGTTCTGTGGGGCATTCACCAC
CCCAATGACGCAGCTGAGCAGACTAAATTGTACCAAAACCCAACTACCTATATATCAGTT
GGTACCTCAACTCTTAACCAGCGACTAGTCCCCCGTATTGCTACTAGGTCAAAGGTTAAT
GGTCAAAGTGGACGAATGGAGTTTTTCTGGACTATTTTGAAGCCCAACGATGCCATCAAC
TTCGAAAGTAATGGAAATTTCATAGCCCCTGAGTACGCTTACAAAATCGTTAAAAAGGGT
GATTCCACTATCATGAAATCTGAACTGGAATACGGAAACTGTAACACCAAATGCCAGACG
CCAATGGGTGCCATCAACTCTTCTATGCCTTTTCACAACATTCATCCTTTGACTATTGGT
GAATGCCCAAAGTACGTCAAATCTAACCGTTTGGTGTTGGCTACTGGTCTAAGGAACTCC
CCTCAGCGTGAAAGAAGAAGAAAGAAGAGGGGATTATTCGGTGCTATCGCTGGATTTATT
GAGGGAGGATGGCAGGGAATGGTCGATGGCTGGTATGGTTACCATCACTCAAATGAACAG
GGAAGTGGATACGCAGCTGATAAAGAATCTACTCAAAAGGCTATCGACGGTGTTACAAAC
AAGGTCAATTCTATTATCGATAAGATGAATACACAGTTTGAGGCTGTTGGTAGAGAGTTC
AATAATCTTGAGAGAAGAATCGAAAACCTGAACAAGAAAATGGAAGACGGATTTTTAGAT
GTATGGACTTACAATGCTGAGTTGTTGGTCTTGATGGAGAATGAACGAACGTTGGACTTC
CATGACTCCAATGTGAAGAACCTATATGACAAAGTGAGGCTGCAACTTAGAGACAACGCC
AAGGAATTGGGAAACGGGTGCTTCGAGTTTTACCACAAATGCGACAACGAATGTATGGAA
TCAGTGAGAAACGGTACCTATGATTACCCCCAATATTCCGAGGAGGCAAGACTGAAGAGA
GAAGAGATATCTGGTGTAAAGTTGGAATCCATCGGTATTTATCAGATTCTATCTATATAT
TCTACCTAATAG

Figure 44

SEQ ID NO: 563 E. coli fliC Amino Acid sequence (without hinge region)

MAQVINTNSLSLITQNNINKNQSALSSSIERLSSGLRINSAKDDAAGQAIANRFTSNIKG
LTQAARNANDGISVAQTTEGALSEINNNLQRIRELTVQASTGTNSDSDLDSIQDEIKSRL
DEIDRVSGQTQFNGVNVLAKDGSMKIQVGANDGQTITIDLKKIDSDTLGTKDPLKALDEA
ISSIDKFRSSLGAIQNRLDSAVTNLNNTTTNLSEAQSRIQDADYATEVSNMSKAQIIQQA
GNSVLAKANQVPQQVLSLLQG

Figure 45

SEQ ID: 585 Amino acid sequence of pMT/STF2Δ

MKLCILLAVVAFVGLSLGRSAQVINTNSLSLLTQNNLNKSQSALGTAIERLS
SGLRINSAKDDAAGQAIANRFTANIKGLTQASRNANDGISIAQTTEGALNEI
NNNLQRVRELAVQSANSTNSQSDLDSIQAEITQRLNEIDRVSGQTQFNGVKV
LAQDNTLTIQVGANDGETIDIDLKQINSQTLGLDSLNVHGAPVDPASPWTEN
PLQKIDAALAQVDALRSDLGAVQNRFNSAITNLGNTVNNLSEARSRIEDSDY
ATEVSNMSRAQILQQAGTSVLAQANQVPQNVLSLLREFSRYPAQWRPLTRTG
HHHHHH

Figure 46

SEQ ID: 586 Nucleic acid sequence of pMT/STF2Δ

ATGAAGTTATGCATATTACTGGCCGTCGTGGCCTTTGTTGGCCTCTCGCTCG
GGAGATCTGCACAAGTAATCAACACTAACAGTCTGTCGCTGCTGACCCAGAA
TAACCTGAACAAATCCCAGTCCGCACTGGGCACCGCTATCGAGCGTCTGTCT
TCTGGTCTGCGTATCAACAGCGCGAAAGACGATGCGGCAGGTCAGGCGATTG
CTAACCGTTTCACCGCGAACATCAAAGGTCTGACTCAGGCTTCCCGTAACGC
TAACGACGGTATCTCCATTGCGCAGACCACTGAAGGCGCGCTGAACGAAATC
AACAACAACCTGCAGCGTGTGCGTGAACTGGCGGTTCAGTCTGCTAACAGCA
CCAACTCCCAGTCTGACCTCGACTCCATCCAGGCTGAAATCACCCAGCGCCT
GAACGAAATCGACCGTGTATCCGGCCAGACTCAGTTCAACGGCGTGAAAGTC
CTGGCGCAGGACAACACCCTGACCATCCAGGTTGGCGCCAACGACGGTGAAA
CTATCGATATCGATCTGAAGCAGATCAACTCTCAGACCCTGGGTCTGGACTC
ACTGAACGTGCATGGAGCGCCGGTGGATCCTGCTAGCCCATGGACCGAAAAC
CCGCTGCAGAAAATTGATGCCGCGCTGGCGCAGGTGGATGCGCTGCGCTCTG
ATCTGGGTGCGGTACAAAACCGTTTCAACTCTGCTATCACCAACCTGGGCAA
TACCGTAAACAATCTGTCTGAAGCGCGTAGCCGTATCGAAGATTCCGACTAC
GCGACCGAAGTTTCCAACATGTCTCGCGCGCAGATTTTGCAGCAGGCCGGTA
CTTCCGTTCTGGCGCAGGCTAACCAGGTCCCGCAGAACGTGCTGTCTCTGTT
ACGTGAATTCTCTAGATATCCAGCACAGTGGCGGCCGCTCACGCGTACCGGT
CATCATCACCATCACCATTGA

Figure 47

SEQ ID NO: 595 Salmonella muenchen fliC Amino Acid Sequence (Hinge region deleted)

MAQVINTNSLSLLTQNNLNKSQSALGTAIERLSSGLRINSAKDDAAGQAIANRFTANIKG
LTQASRNANDGISIAQTTEGALNEINNNLQRVRELAVQSANGTNSQSDLDSIQAEITQRL
NEIDRVSGQTQFNGVKVLAQDNTLTIQVGANDGETIDIDLKEISSKTLDKHNFRTGGELK
EVNTDKTENPLQKIDAALAQVDTLRSDLGAVQNRFNSAITNLGNTVNNLSSARSRIEDSD
YATEVSNMSRAQILQQAGTSVLAQANQVPQNVLSLLR

Figure 48

SEQ ID NO: 596 Salmonella Muenchen fliC Nucleic Acid Sequence (Hinge region deleted)

ATGGCACAAGTCATTAATACAAACAGCCTGTCGCTGTTGACCCAGAATAACCTGAACAAA
TCCCAGTCCGCTCTGGGCACCGCTATCGAGCGTCTGTCTTCCGGTCTGCGTATCAACAGC
GCGAAAGACGATGCGGCAGGTCAGGCGATTGCTAACCGTTTCACCGCGAACATCAAAGGT
CTGACTCAGGCTTCCCGTAACGCTAACGACGGTATCTCCATTGCGCAGACCACTGAAGGC
GCGCTGAACGAAATCAACAACAACCTGCAGCGTGTGCGTGAACTGGCGGTTCAGTCTGCT
AACGGTACTAACTCCCAGTCTGACCTTGACTCTATCCAGGCTGAAATCACCCAGCGTCTG
AACGAAATCGACCGTGTATCCGGTCAGACTCAGTTCAACGGCGTGAAAGTCCTGGCGCAG
GACAACACCCTGACCATCCAGGTTGGTGCCAACGACGGTGAAACTATTGATATTGATTTA
AAAGAAATTAGCTCTAAAACACTGACAGATAAGACTGAAAACCCACTGCAGAAAATTGAT
GCTGCCTTGGCACAGGTTGATACACTTCGTTCTGACCTGGGTGCGGTACAGAACCGTTTC
AACTCCGCTATCACCAACCTGGGCAATACCGTAAATAACCTGTCTTCTGCCCGTAGCCGT
ATCGAAGATTCCGACTACGCGACCGAAGTCTCCAACATGTCTCGCGCGCAGATTCTGCAG
CAGGCCGGTACCTCCGTTCTGGCGCAGGCTAACCAGGTTCCGCAAAACGTCCTCTCTTTA
CTGCGTTAA

Figure 49

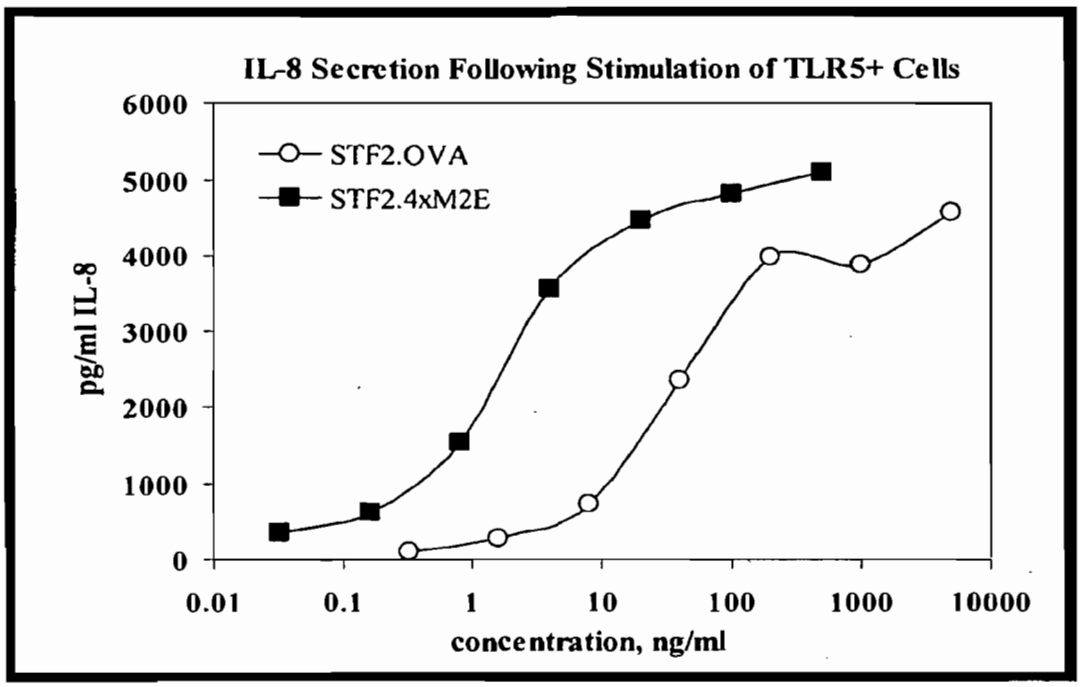

IL-8 Secretion Following Stimulation of TLR5+ Cells
6000
5000
4000
3000
2000
1000
0
pg/ml IL-8
STF2.OVA
STF2.4xM2E
0.01
0.1
1
10
100
1000
10000
concentration, ng/ml

Figure 50

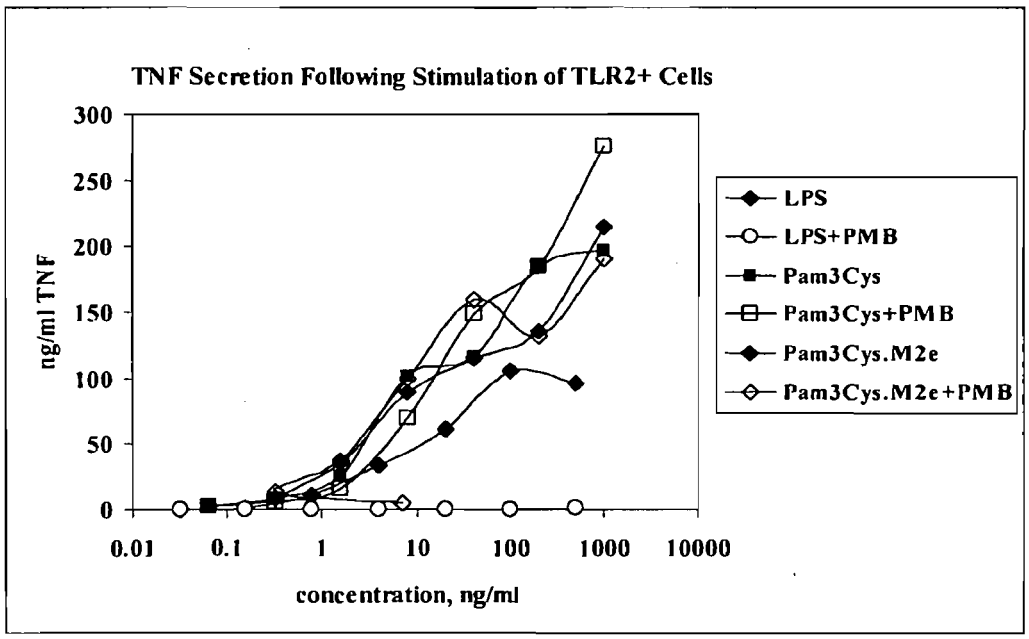

TNF Secretion Following Stimulation of TLR2+ Cells
ng/ml TNF
300
250
200
150
100
50
0
0.01
0.1
1
10
100
1000
10000
concentration, ng/ml
LPS
LPS+PMB
Pam3Cys
Pam3Cys+PMB
Pam3Cys.M2e
Pam3Cys.M2e+PMB

Figure 51

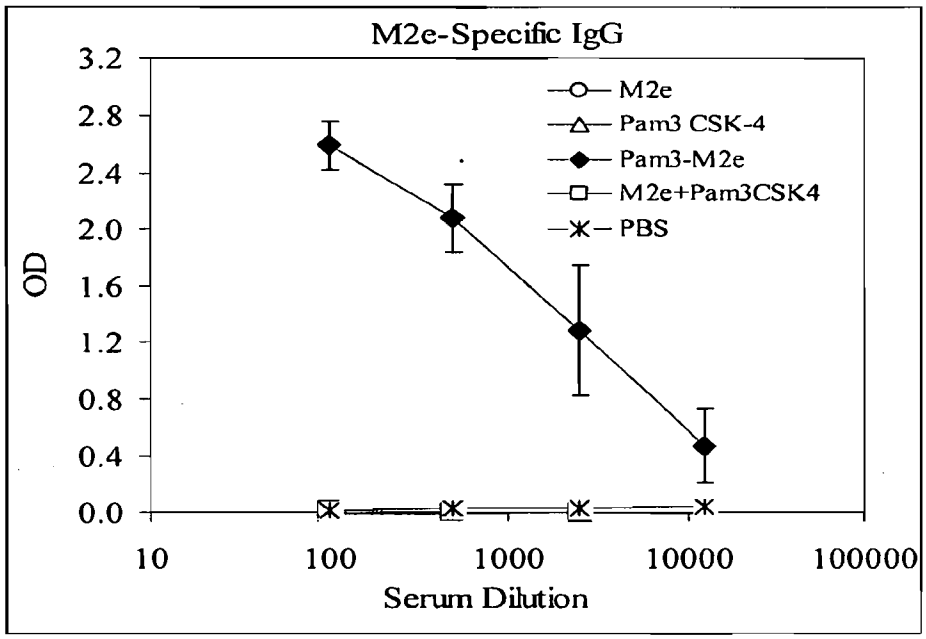

M2e-Specific IgG
3.2
2.8
2.4
2.0
1.6
1.2
0.8
0.4
0.0
OD
M2e
Pam3 CSK-4
Pam3-M2e
M2e+Pam3CSK4
PBS
10
100
1000
10000
100000
Serum Dilution

Figure 52

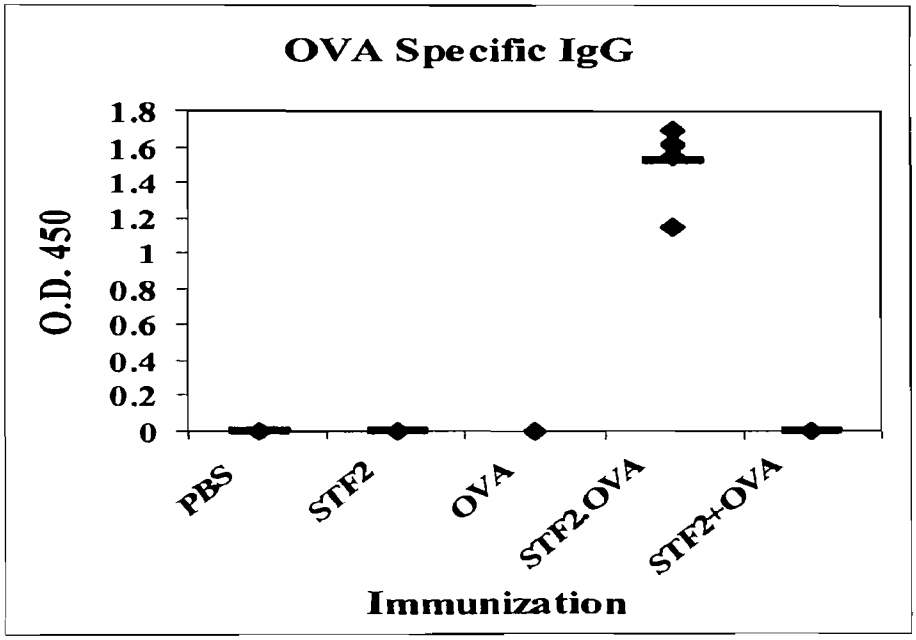

OVA Specific IgG
O.D. 450
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
PBS
STF2
OVA
STF2OVA
STF2+OVA
Immunization

Figure 53

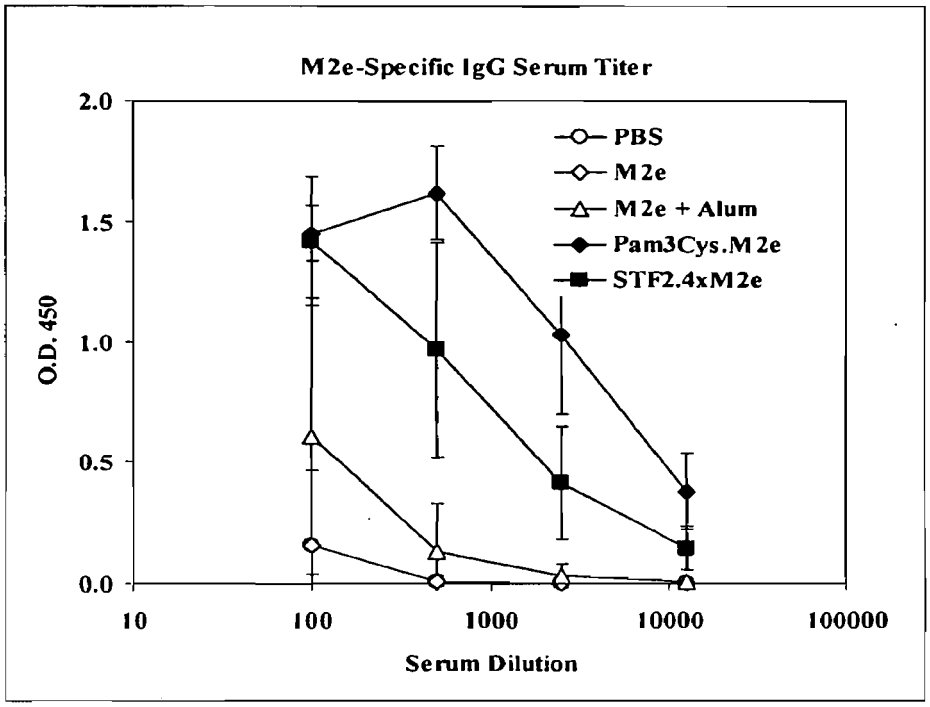

M2e-Specific IgG Serum Titer
2.0
1.5
1.0
0.5
0.0
O.D. 450
10
100
1000
10000
100000
Serum Dilution
PBS
M2e
M2e + Alum
Pam3Cys.M2e
STF2.4xM2e

Figure 54

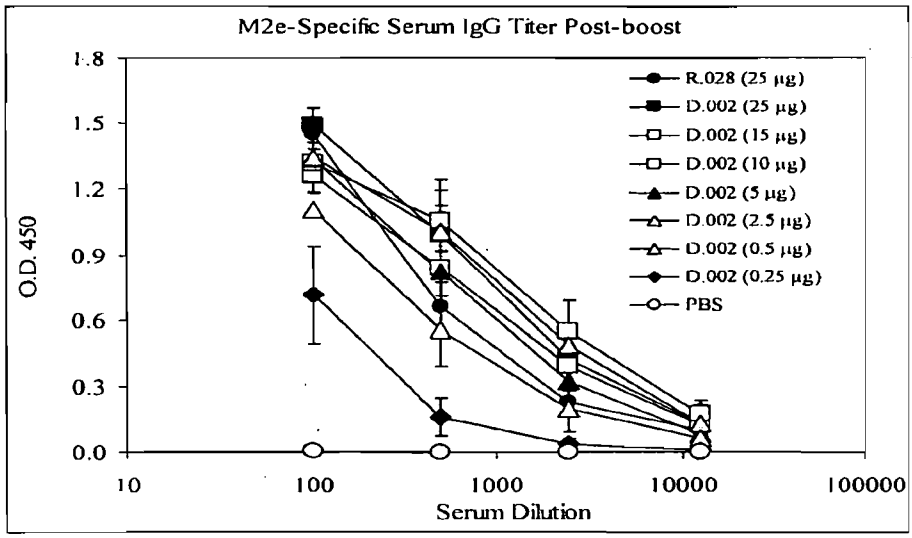

M2e-Specific Serum IgG Titer Post-boost
O.D.450
1.8
1.5
1.2
0.9
0.6
0.3
0.0
10
100
1000
10000
100000
Serum Dilution
R.028 (25 µg)
D.002 (25 µg)
D.002 (15 µg)
D.002 (10 µg)
D.002 (5 µg)
D.002 (2.5 µg)
D.002 (0.5 µg)
D.002 (0.25 µg)
PBS

Figure 55

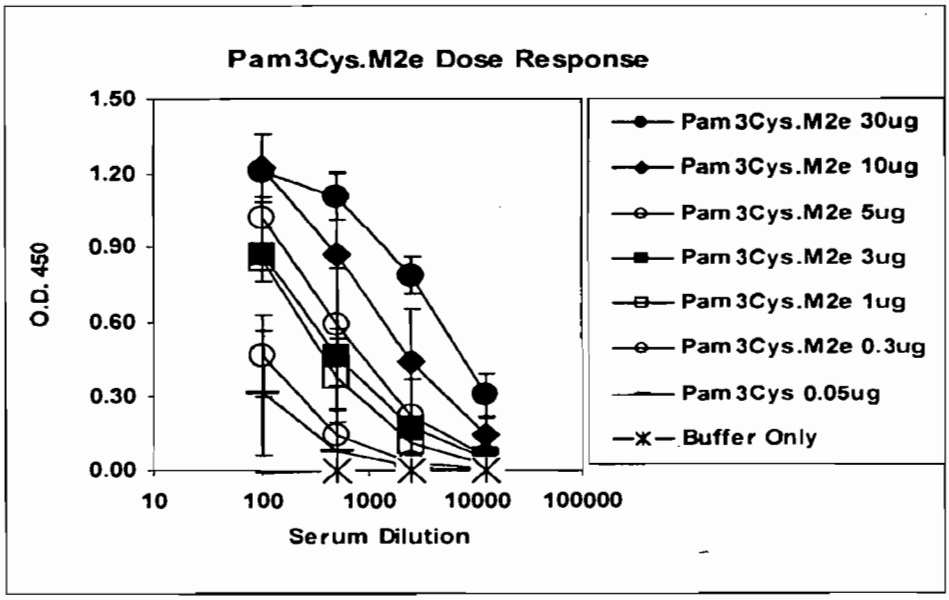

Pam3Cys.M2e Dose Response
1.50
1.20
0.90
0.60
0.30
0.00
O.D. 450
10
100
1000
10000
100000
Serum Dilution
Pam3Cys.M2e 30ug
Pam3Cys.M2e 10ug
Pam3Cys.M2e 5ug
Pam3Cys.M2e 3ug
Pam3Cys.M2e 1ug
Pam3Cys.M2e 0.3ug
Pam3Cys 0.05ug
Buffer Only

Figure 56

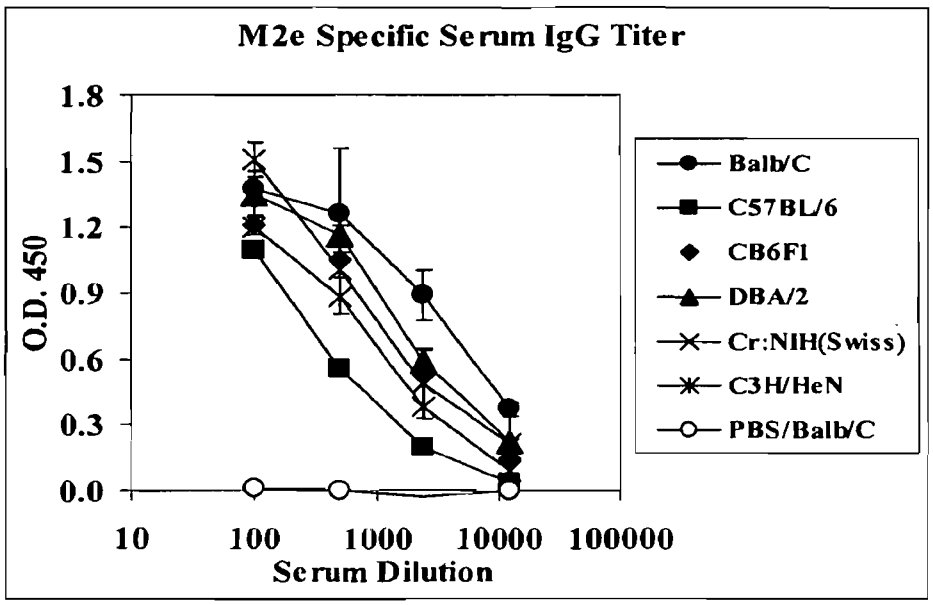

M2e Specific Serum IgG Titer
1.8
1.5
1.2
0.9
0.6
0.3
0.0
O.D. 450
10
100
1000
10000
100000
Serum Dilution
Balb/C
C57BL/6
CB6F1
DBA/2
Cr:NIH(Swiss)
C3H/HeN
PBS/Balb/C

Figure 57

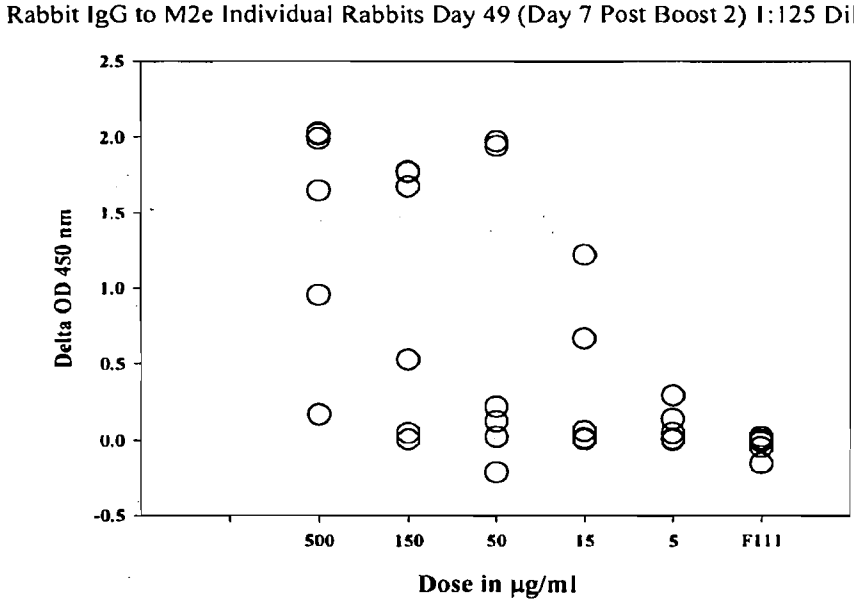

Rabbit IgG to M2e Individual Rabbits Day 49 (Day 7 Post Boost 2) 1:125 Dilution
Delta OD 450 nm
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
500
150
50
15
5
F111
Dose in µg/ml

Figure 58

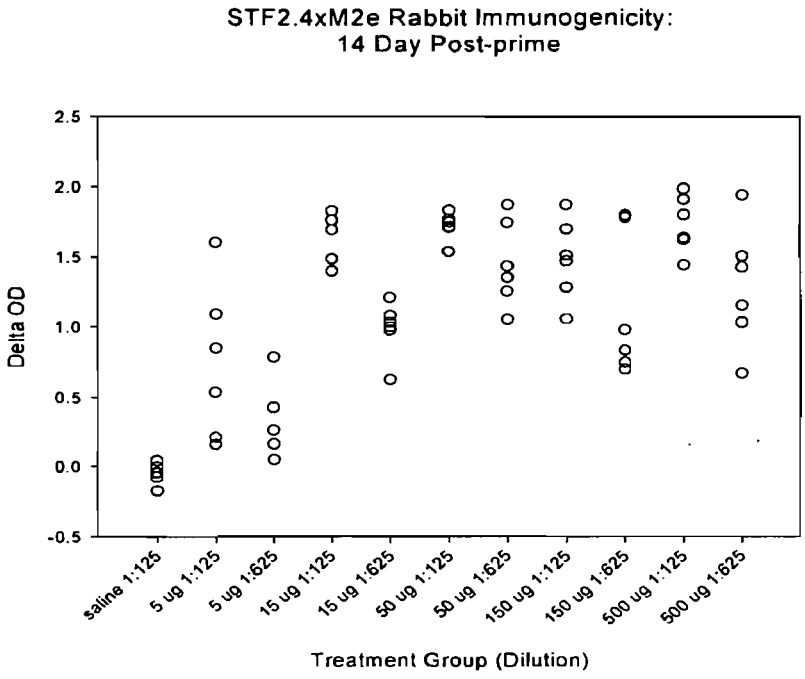

STF2.4xM2e Rabbit Immunogenicity:
14 Day Post-prime
Delta OD
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
saline 1:125
5 ug 1:125
5 ug 1:625
15 ug 1:125
15 ug 1:625
50 ug 1:125
50 ug 1:625
150 ug 1:125
150 ug 1:625
500 ug 1:125
500 ug 1:625
Treatment Group (Dilution)

Figure 59

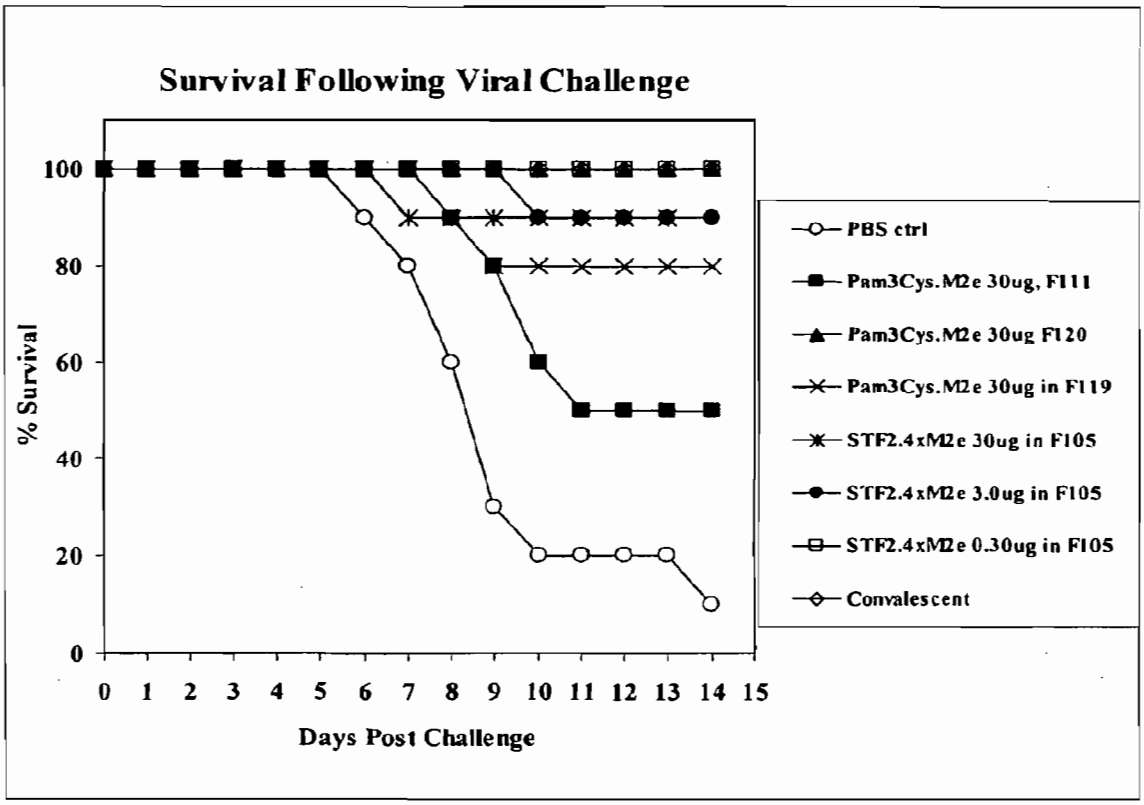

Survival Following Viral Challenge
% Survival
100
80
60
40
20
0
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
Days Post Challenge
PBS ctrl
Pam3Cys.M2e 30ug, F111
Pam3Cys.M2e 30ug F120
Pam3Cys.M2e 30ug in F119
STF2.4xM2e 30ug in F105
STF2.4xM2e 3.0ug in F105
STF2.4xM2e 0.30ug in F105
Convalescent

Figure 60


T7
lacO
STF2
EIII+
pET/STF2.EIII+
T7
lacO
STF2Δ
EIII+
pET/STF2ΔEIII+

Figure 61

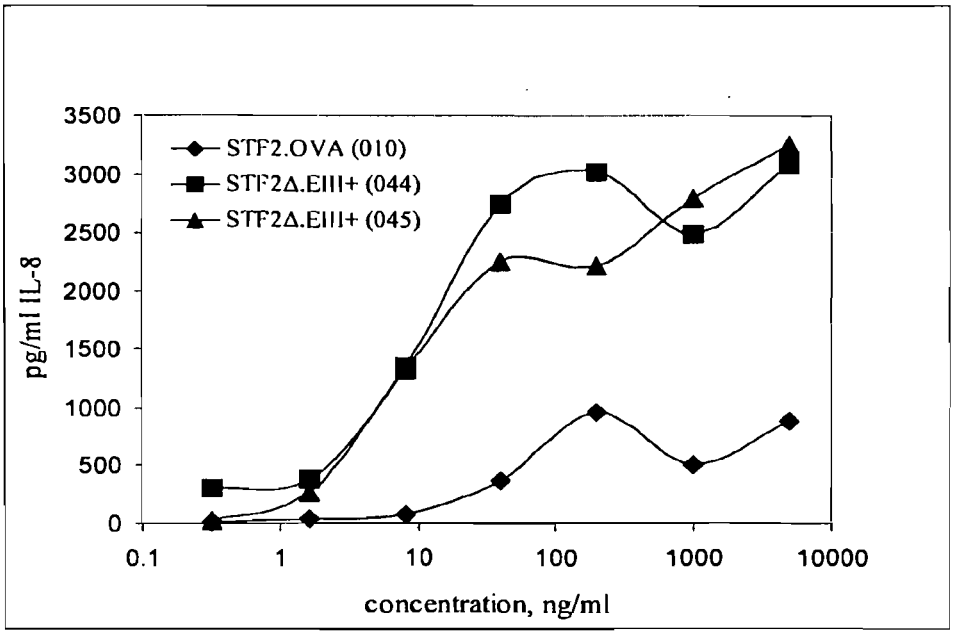

3500
3000
2500
2000
1500
1000
500
0
pg/ml IL-8
STF2.OVA (010)
STF2Δ.EIII+ (044)
STF2Δ.EIII+ (045)
0.1
1
10
100
1000
10000
concentration, ng/ml

Figure 62A

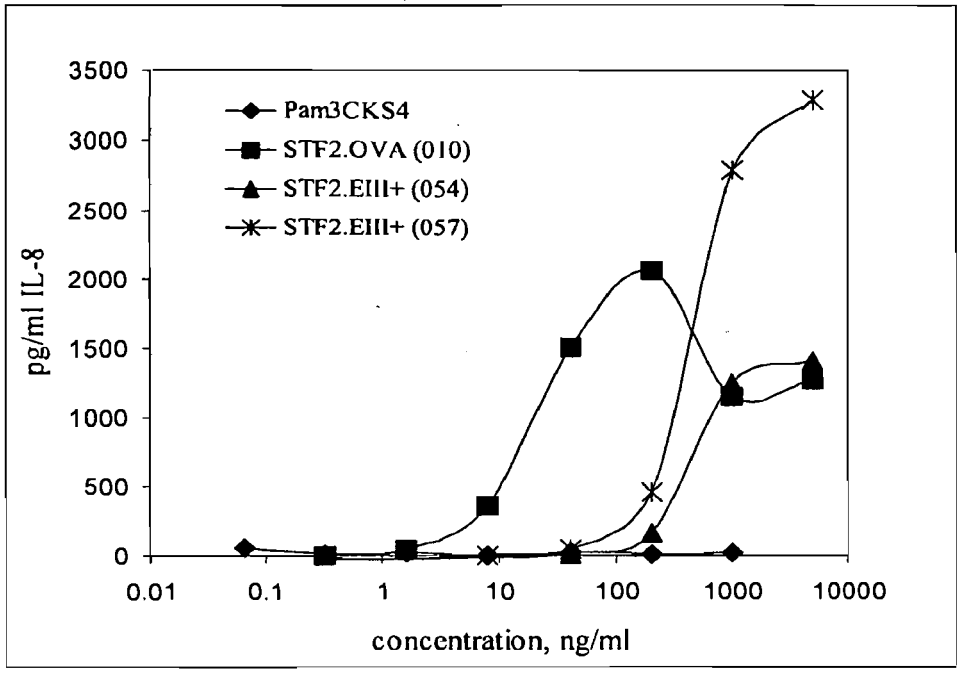

3500
3000
2500
2000
1500
1000
500
0
pg/ml IL-8
Pam3CKS4
STF2.OVA (010)
STF2.EIII+ (054)
STF2.EIII+ (057)
0.01
0.1
1
10
100
1000
10000
concentration, ng/ml

Figure 62B

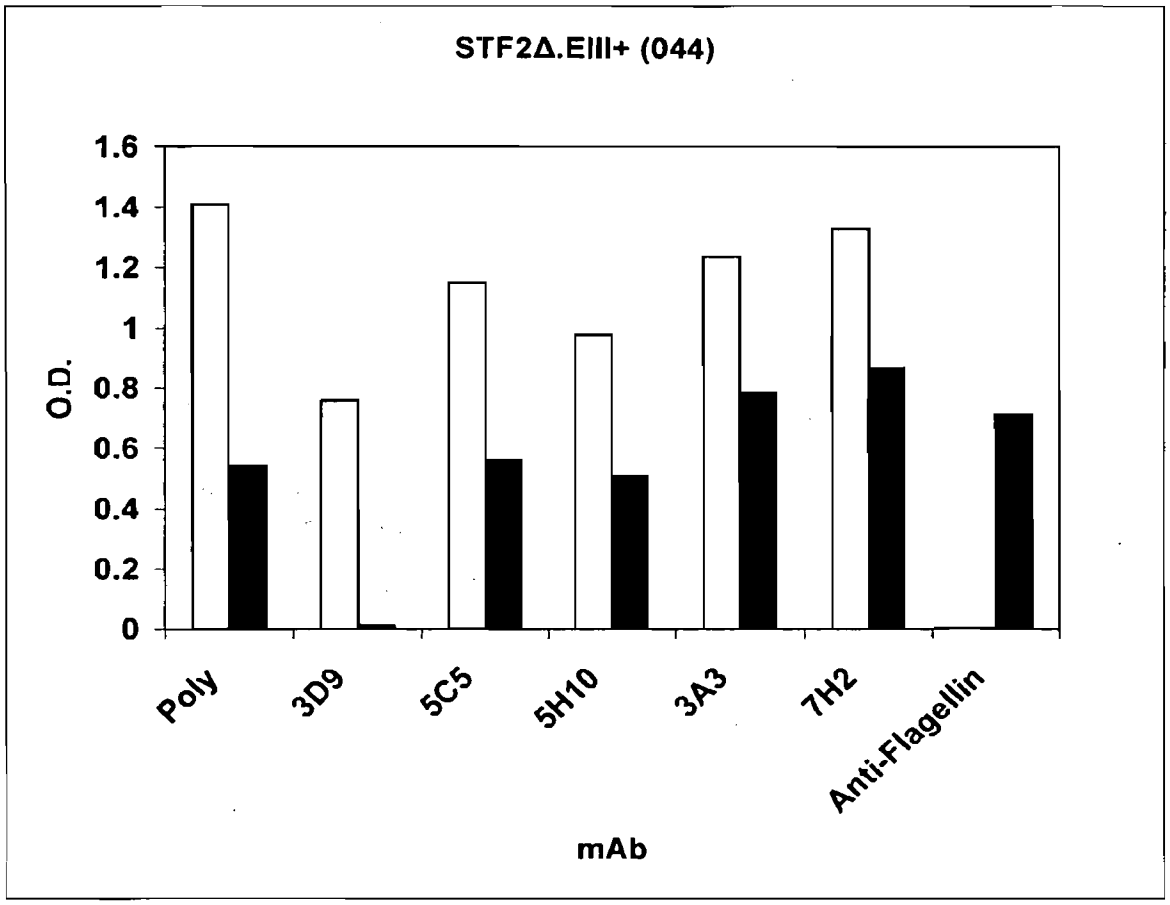

STF2Δ.EIII+ (044)
O.D.
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
Poly
3D9
5C5
5H10
3A3
7H2
Anti-Flagellin
mAb

Figure 63

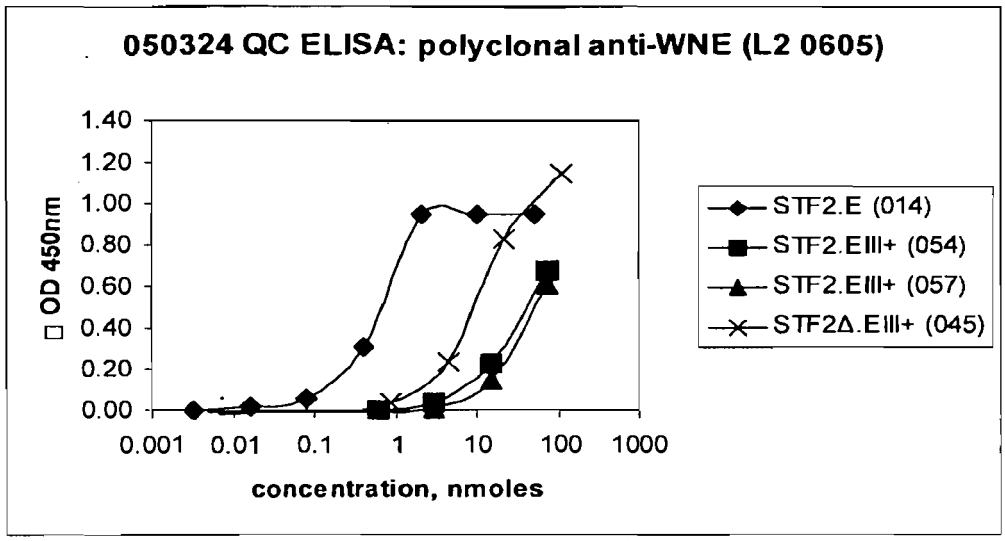

050324 QC ELISA: polyclonal anti-WNE (L2 0605)
OD 450nm
1.40
1.20
1.00
0.80
0.60
0.40
0.20
0.00
0.001
0.01
0.1
1
10
100
1000
concentration, nmoles
STF2.E (014)
STF2.EIII+ (054)
STF2.EIII+ (057)
STF2Δ.EIII+ (045)

Figure 64A

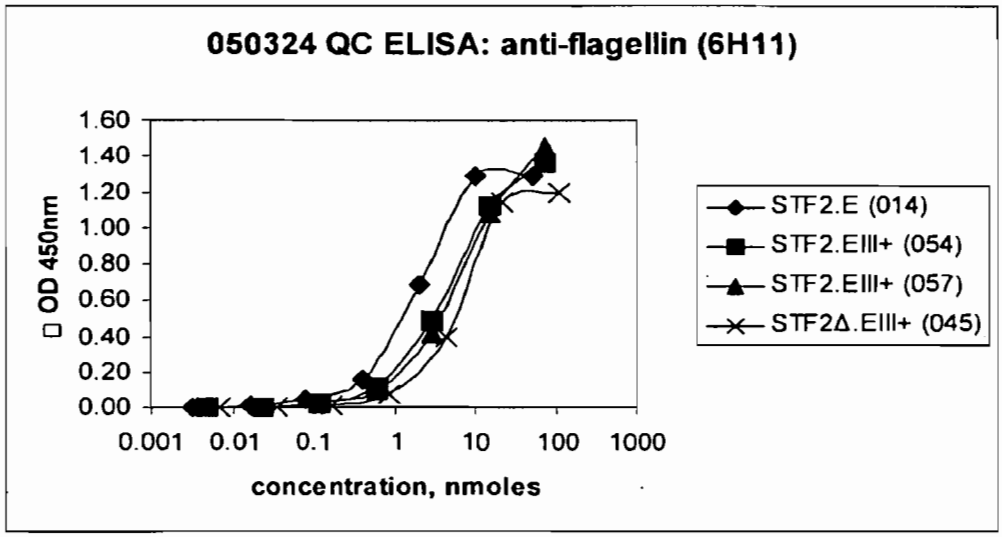

050324 QC ELISA: anti-flagellin (6H11)
OD 450nm
1.60
1.40
1.20
1.00
0.80
0.60
0.40
0.20
0.00
0.001
0.01
0.1
1
10
100
1000
concentration, nmoles
STF2.E (014)
STF2.EIII+ (054)
STF2.EIII+ (057)
STF2Δ.EIII+ (045)

Figure 64B

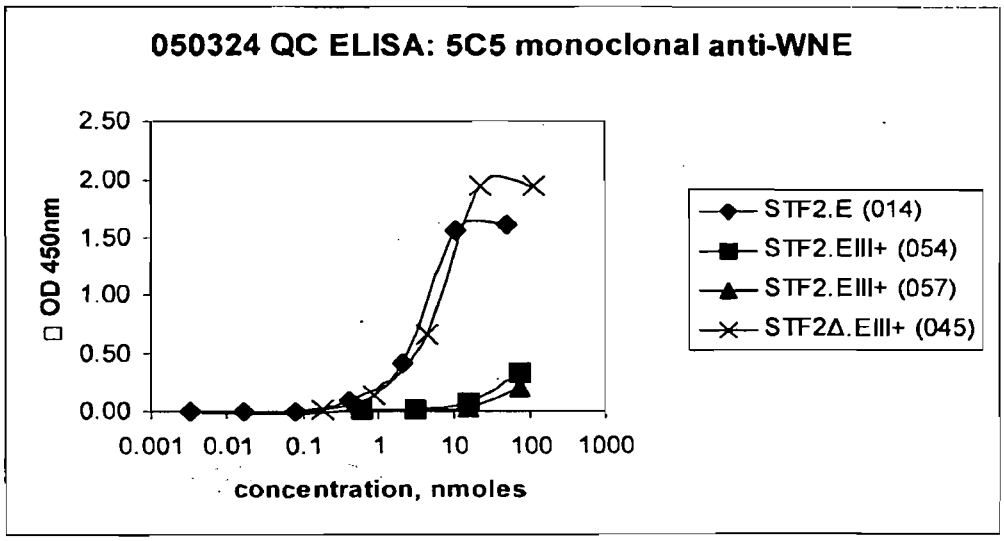

050324 QC ELISA: 5C5 monoclonal anti-WNE
OD 450nm
2.50
2.00
1.50
1.00
0.50
0.00
0.001
0.01
0.1
1
10
100
1000
concentration, nmoles
STF2.E (014)
STF2.EIII+ (054)
STF2.EIII+ (057)
STF2Δ.EIII+ (045)

Figure 64C

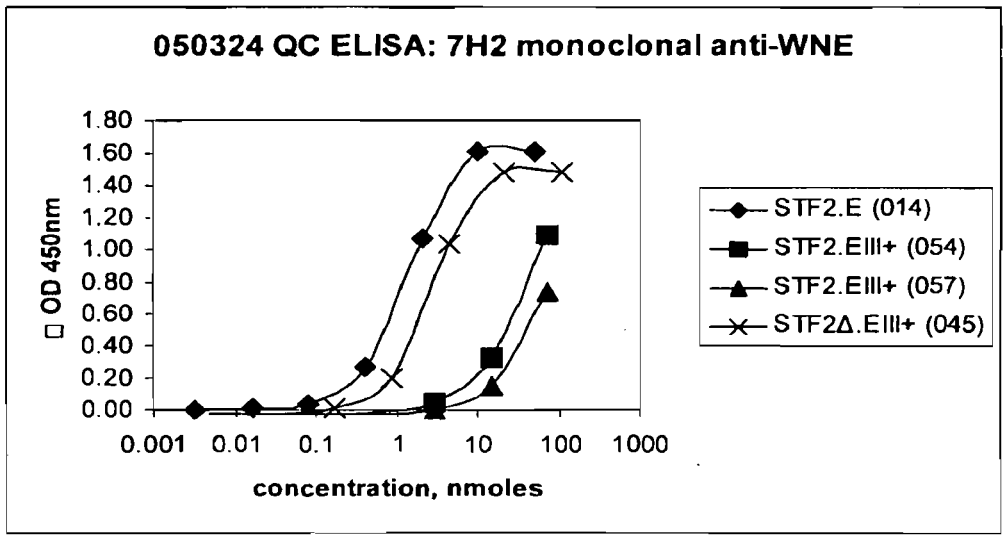

050324 QC ELISA: 7H2 monoclonal anti-WNE
OD 450nm
1.80
1.60
1.40
1.20
1.00
0.80
0.60
0.40
0.20
0.00
0.001
0.01
0.1
1
10
100
1000
concentration, nmoles
STF2.E (014)
STF2.EIII+ (054)
STF2.EIII+ (057)
STF2Δ.EIII+ (045)

Figure 64D

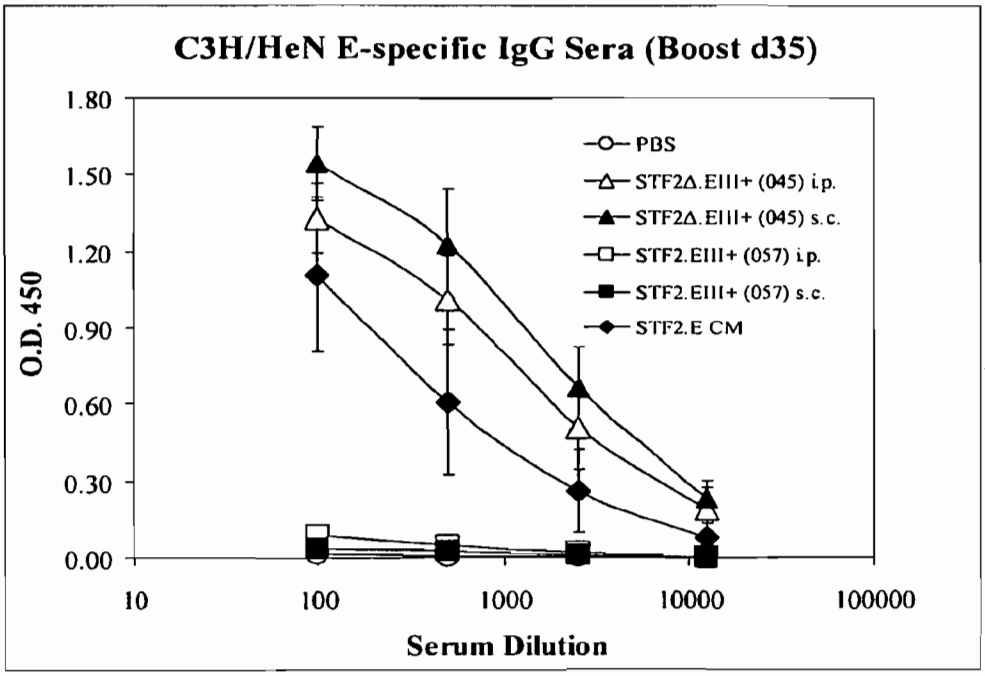

C3H/HeN E-specific IgG Sera (Boost d35)
O.D. 450
1.80
1.50
1.20
0.90
0.60
0.30
0.00
10
100
1000
10000
100000
Serum Dilution
PBS
STF2Δ.EIII+ (045) i.p.
STF2Δ.EIII+ (045) s.c.
STF2.EIII+ (057) i.p.
STF2.EIII+ (057) s.c.
STF2.E CM

Figure 65

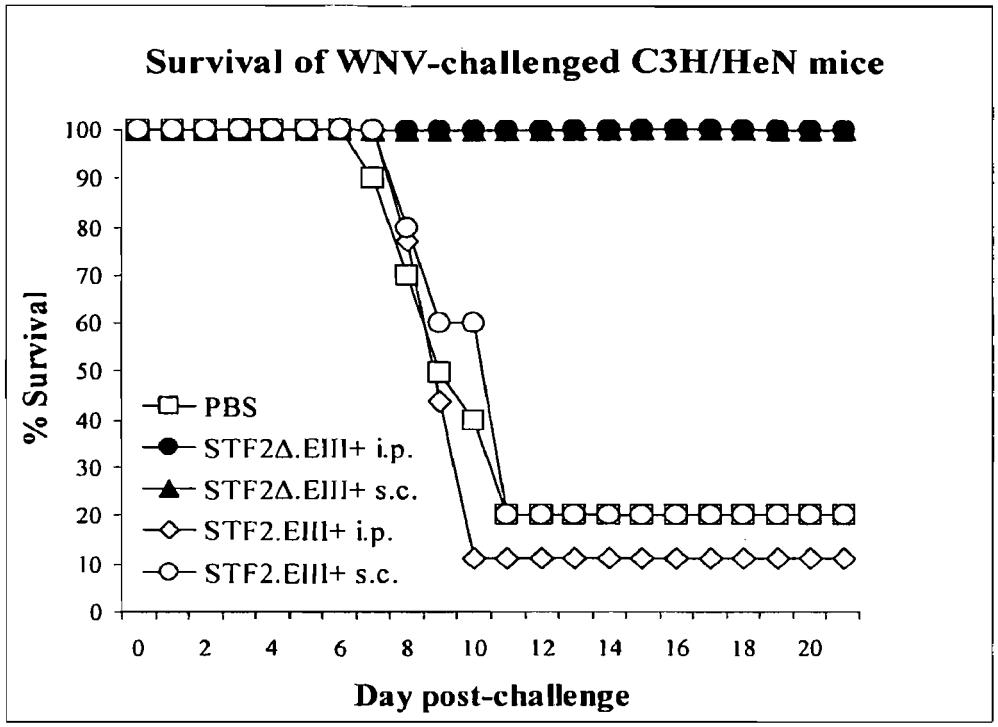

Survival of WNV-challenged C3H/HeN mice
% Survival
100
90
80
70
60
50
40
30
20
10
0
0
2
4
6
8
10
12
14
16
18
20
Day post-challenge
PBS
STF2Δ.EIII+ i.p.
STF2Δ.EIII+ s.c.
STF2.EIII+ i.p.
STF2.EIII+ s.c.

Figure 66

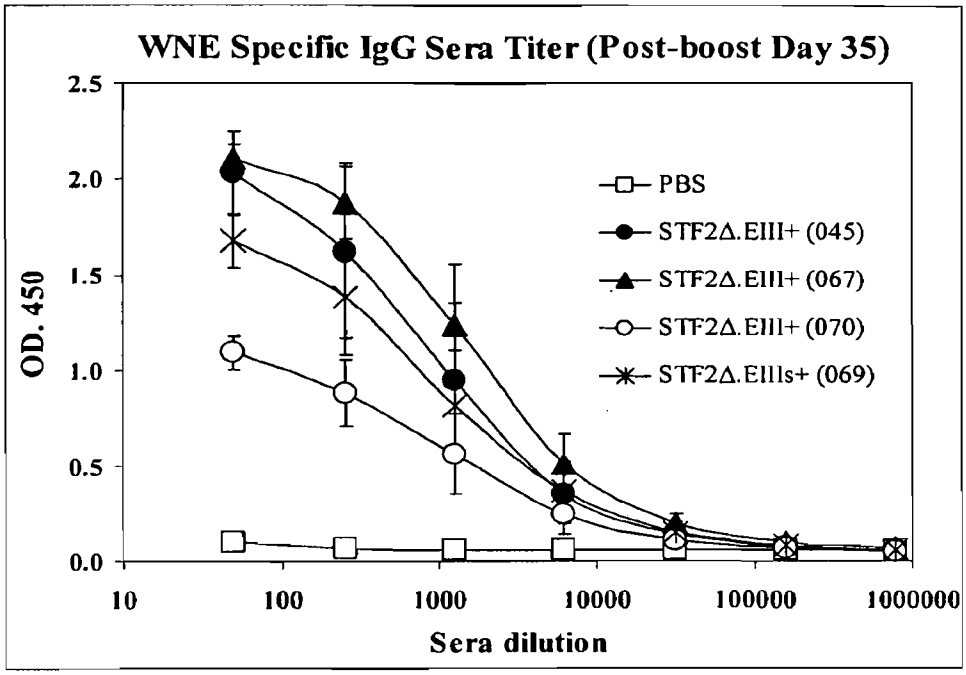

WNE Specific IgG Sera Titer (Post-boost Day 35)
OD. 450
2.5
2.0
1.5
1.0
0.5
0.0
10
100
1000
10000
100000
1000000
Sera dilution
PBS
STF2Δ.EIII+ (045)
STF2Δ.EIII+ (067)
STF2Δ.EIII+ (070)
STF2Δ.EIIIs+ (069)

Figure 67

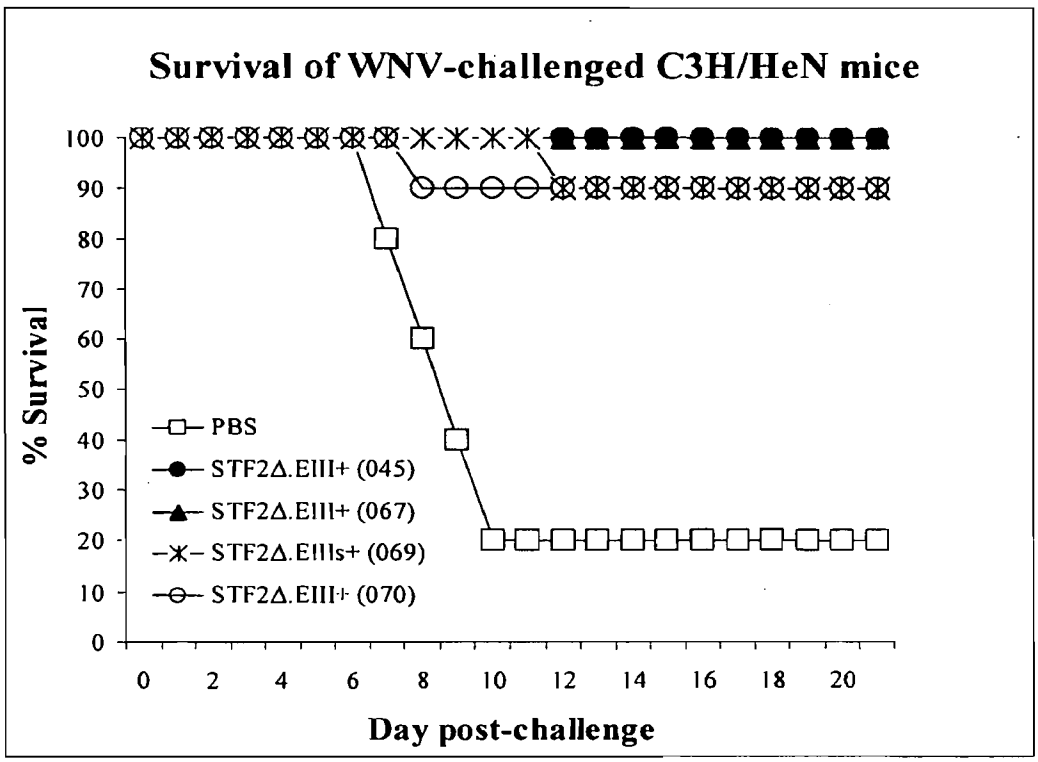

Survival of WNV-challenged C3H/HeN mice
% Survival
100
90
80
70
60
50
40
30
20
10
0
0
2
4
6
8
10
12
14
16
18
20
Day post-challenge
PBS
STF2Δ.EIII+ (045)
STF2Δ.EIII+ (067)
STF2Δ.EIIIs+ (069)
STF2Δ.EIII+ (070)

Figure 68

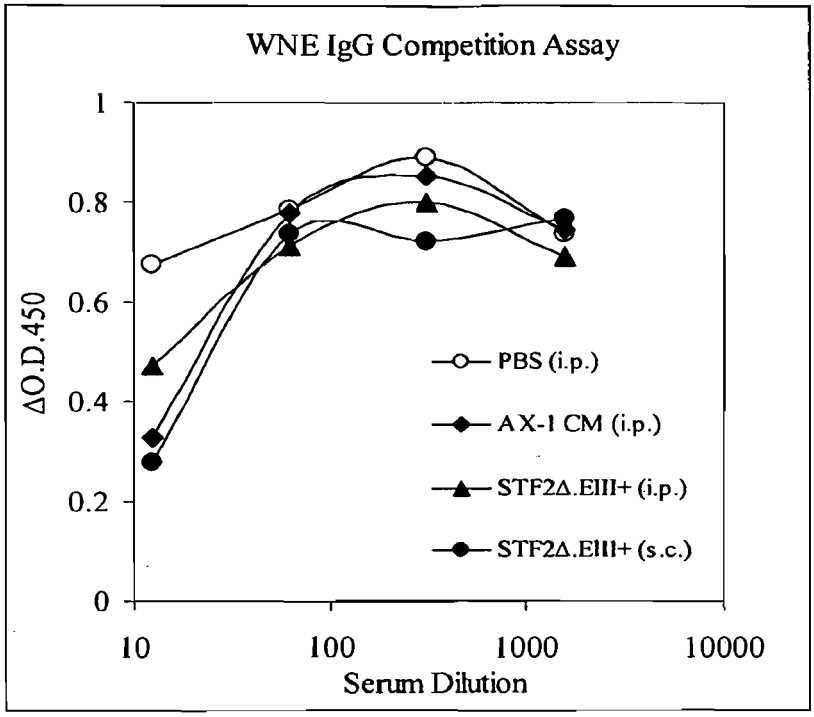

WNE IgG Competition Assay
ΔO.D.450
1
0.8
0.6
0.4
0.2
0
10
100
1000
10000
Serum Dilution
PBS (i.p.)
AX-1 CM (i.p.)
STF2Δ.EIII+ (i.p.)
STF2Δ.EIII+ (s.c.)

Figure 69

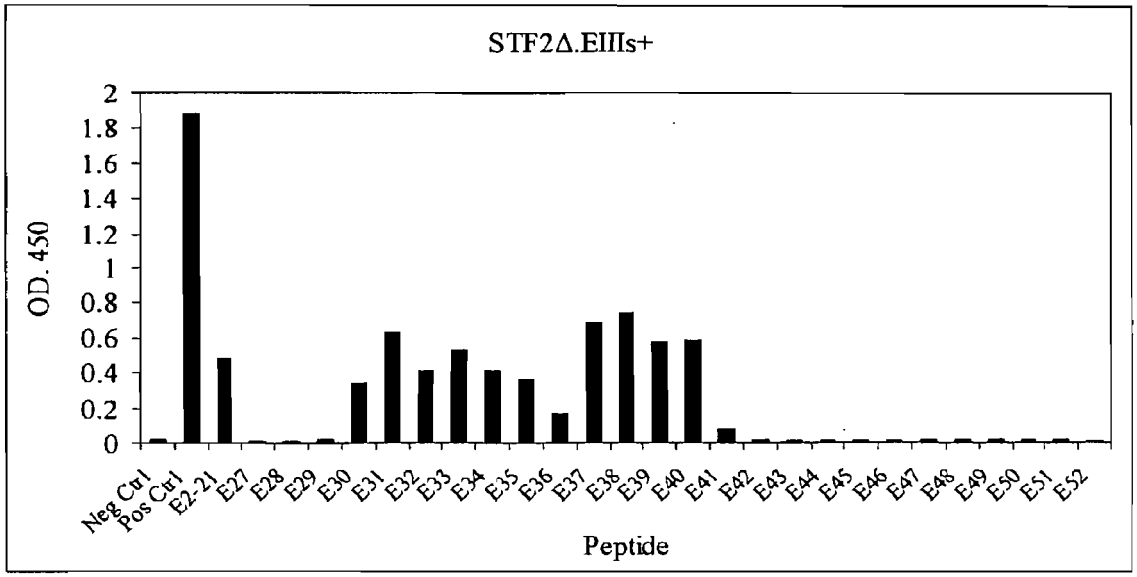

STF2Δ.EIIIs+
OD. 450
2
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
Neg Ctrl
Pos Ctrl
E2-21
E27
E28
E29
E30
E31
E32
E33
E34
E35
E36
E37
E38
E39
E40
E41
E42
E43
E44
E45
E46
E47
E48
E49
E50
E51
E52
Peptide

Figure 70

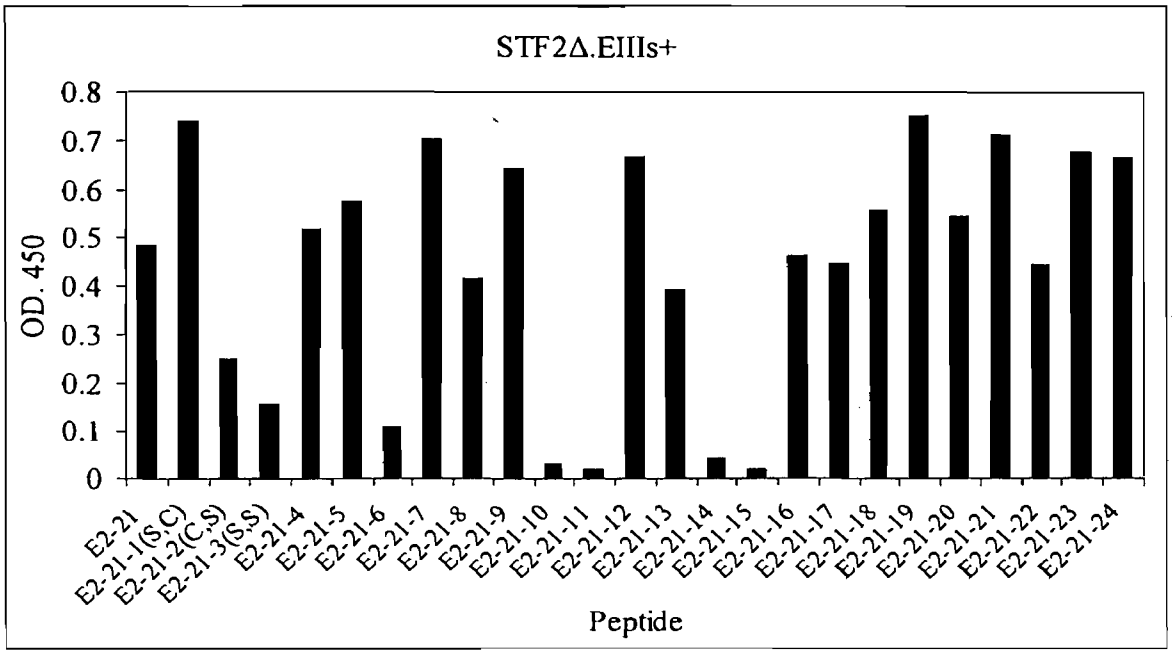

STF2Δ.EIIIs+
OD. 450
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
E2-21
E2-21-1(S,C)
E2-21-2(C,S)
E2-21-3(S,S)
E2-21-4
E2-21-5
E2-21-6
E2-21-7
E2-21-8
E2-21-9
E2-21-10
E2-21-11
E2-21-12
E2-21-13
E2-21-14
E2-21-15
E2-21-16
E2-21-17
E2-21-18
E2-21-19
E2-21-20
E2-21-21
E2-21-22
E2-21-23
E2-21-24
Peptide

Figure 71

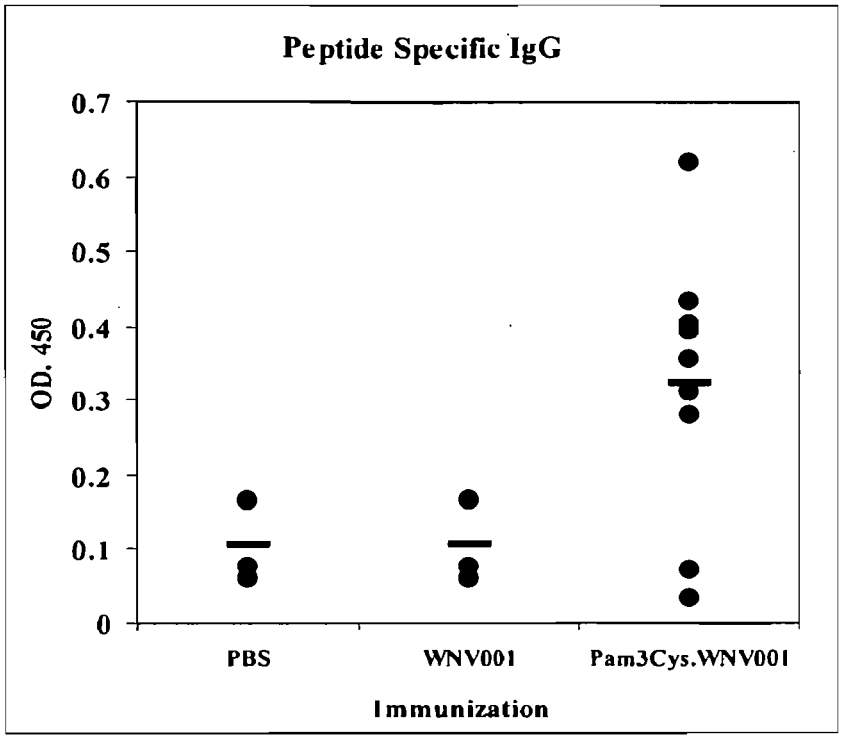

Peptide Specific IgG
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
OD. 450
PBS
WNV001
Pam3Cys.WNV001
Immunization

Figure 72

Flavivirus Envelope Protein Domain I/III junction
(domain I 280-297, domain III 298-406)

WN aa 281-307

WN	LTSGHLKCRVKMEKLQLKGTTYGVCSK	(SEQ ID NO: 691)
JE	LTSGHLKCRLKMDKLALKGTTYGMCTE	(SEQ ID NO: 692)
D1	IFAGHLKCRLKMDKLTLKGMSYVMCTG	(SEQ ID NO: 639)
D2	LFTGHLKCRLRMDKLQLKGMSYSMCTG	(SEQ ID NO: 694)
D3	IFAGHLKCRLKMDKLKLKGMSYAMCLN	(SEQ ID NO: 695)
D4	MFAGHLKCKVRMEKLRIKGMSYTMCSG	(SEQ ID NO: 696)
	xxxGHLKCRxxMxKLxLKGxxYxxCxx	(SEQ ID NO: 697)
	GHLKCRMKLLKGYC	(SEQ ID NO: 698)

Figure 73

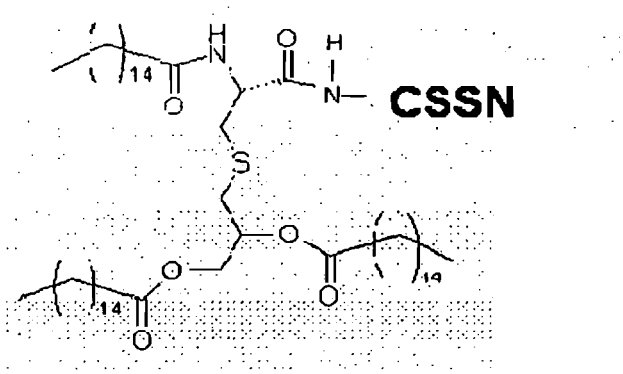


Figure 74

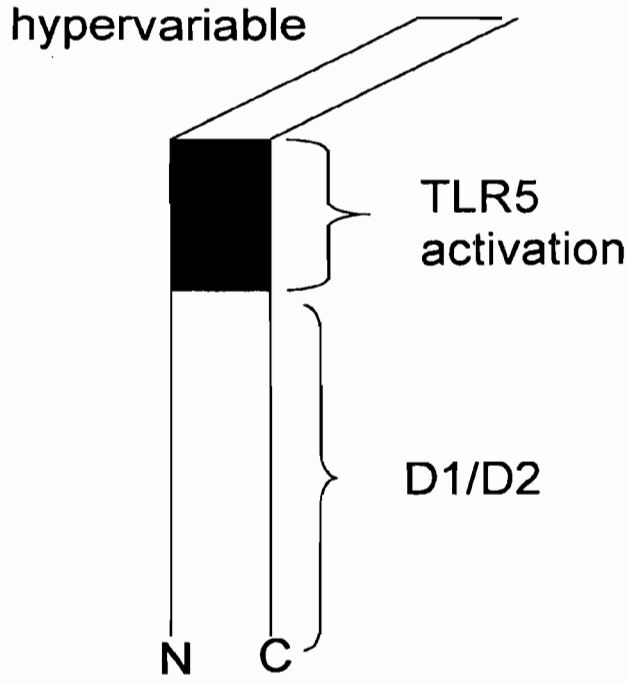


Figure 75

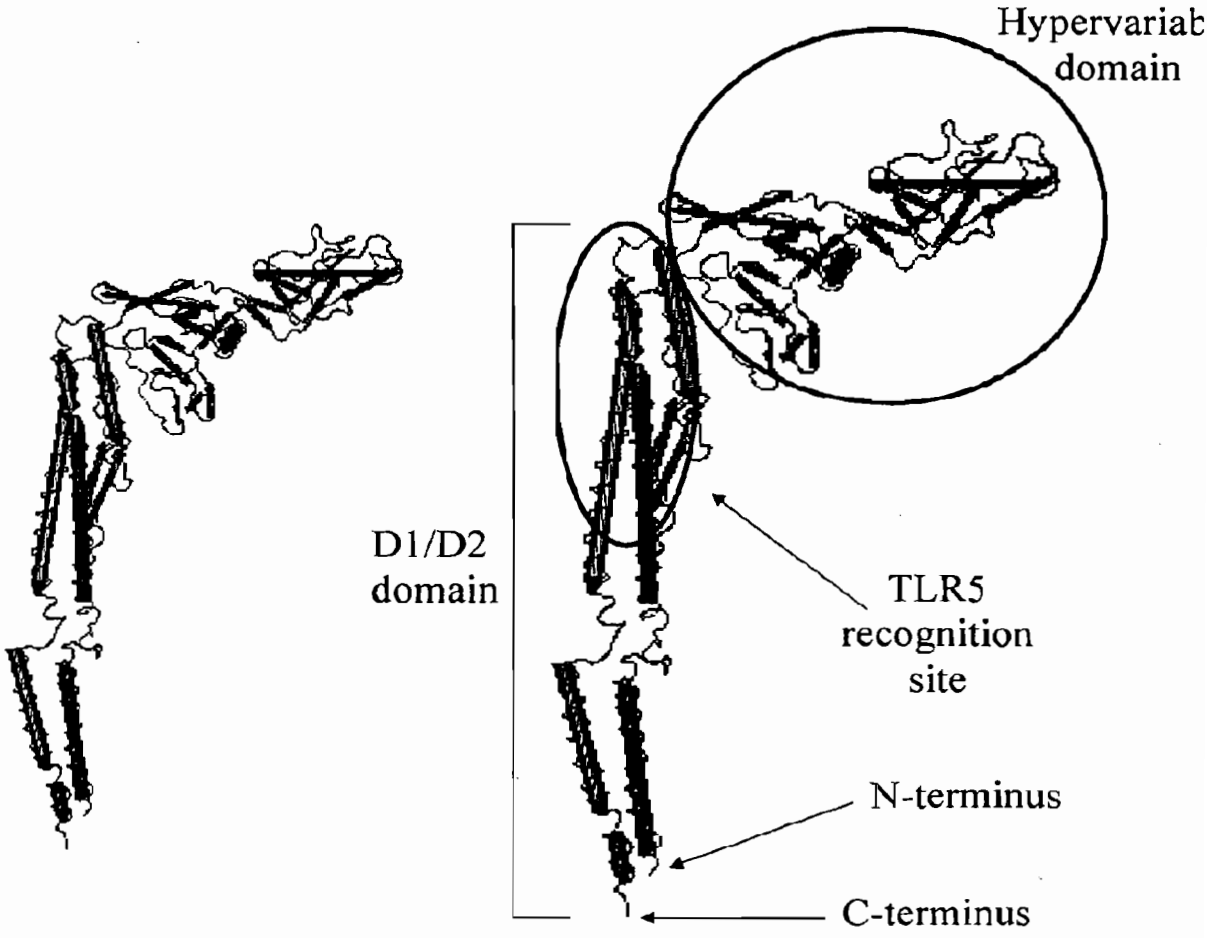
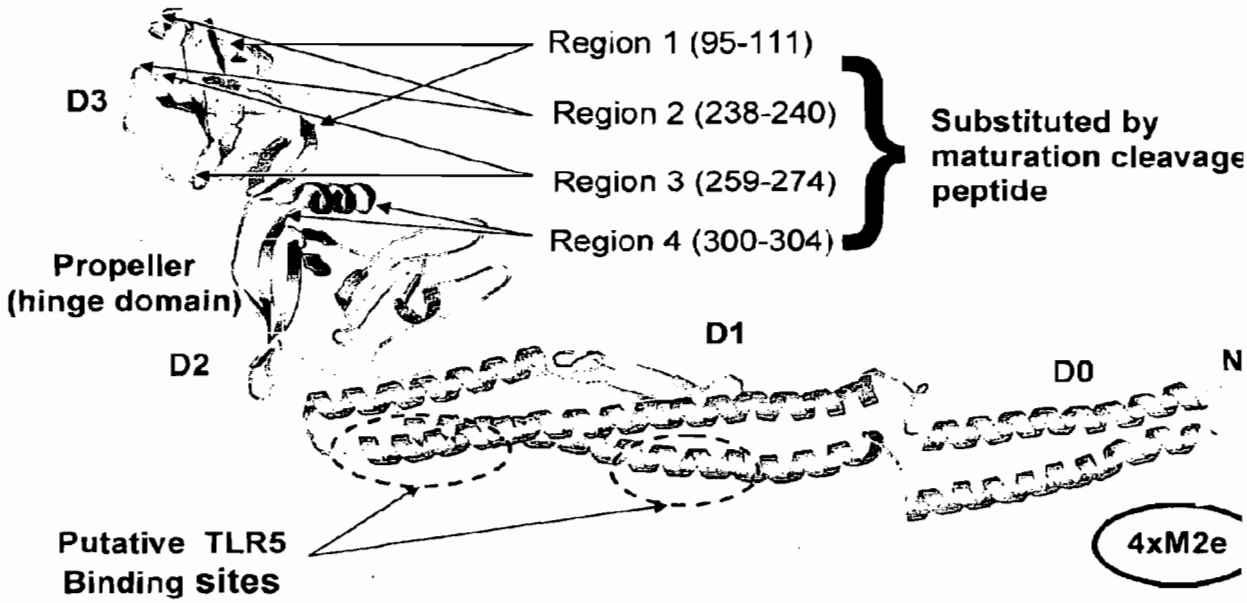

Hypervariab
domain
D1/D2
domain
TLR5
recognition
site
N-terminus
C-terminus

Figure 76



Flagellin (1UCU)

Figure 77

Flagellin from Pseudomonas aeruginosa (accession number P21184)

```
        10          20          30          40 *        50          60
ALTVNTNIAS LNTQRNLNNS SASLNTSLQR LSTGSRINSA KDDAAGLQIA NRLTSQVNGL

    *   70          80          90         100         110       *   *
NVATKNANDG ISLAQTAEGA LQQSTNILQR MRDLSLQSAN GSNSDSERTA LNGEVKQLQK

       130      *  140         150         160         170     *   180
ELDRISNTTT FGGRKLLDGS FGVASFQVGS AANEIISVGI DEMSAESLNG TYFKADGGGA

       190         200      *   *           *         230         240
VTAATASGTV DIAIGITGGS AVNVKVDMKG NETAEQAAAK IAAAVNDANV GIGAFSDGDT

     *  *          260         270         280        *290     *   300
ISYVSKAGKD GSGAITSAVS GVVIADTGST GVGTAAGVTP SATAFAKTND TVAKIDISTA

*       310      *  320         330         *          350         360
KGAQSAVLVI DEAIKQIDAQ RADLGAVQNR FDNTINNLKN IGENVSAARG RIEDTDFAAE

     * 370         380         390
TANLTKNQVL QQAGTAILAQ ANQLPQSVLS LLR
```

Figure 78

STF2 sequence

```
        10          *          30          40  *       50          *
MAQVINTNSL SLLTQNNLNK SQSALGTAIE RLSSGLRINS AKDDAAGQAI ANRFTANIKG

        70          80          90         100         110         120
LTQASRNAND GISIAQTTEG ALNEINNNLQ RVRELAVQSA NSTNSQSDLD SIQAEITQRL

       130       * 140         150         160 *       170        *180
NEIDRVSGQT QFNGVKVLAQ DNTLTIQVGA NDGETIDIDL KQINSQTLGL DSLNVQKAYD

  *       *         200          *         220        *        *   240
VKDTAVTTKA YANNGTTLDV SGLDDAAIKA ATGGTNGTAS VTGGAVKFDA DNNKYFVTIG

        *          260         270 *       280 *       *           *
GFTGADAAKN GDYEVNVATD GTVTLAAGAT KTTMPAGATT KTEVQELKDT PAVVSADAKN

       310          *       *  *          *340 *       350      * *360
ALIAGGVDAT DANGAELVKM SYTDKNGKTI EGGYALKAGD KYYAADYDEA TGAIKAKTTS

        *              *         *       * 400    *    410     *   420
YTAADGTTKT AANQLGGVDG KTEVVTIDGK TYNASKAAGH DFKAQPELAE AAAKTTENPL

  *     430         440         450         460         470         480
QKIDAALAQV DALRSDLGAV QNRFNSAITN LGNTVNNLSE ARSRIEDSDY ATEVSNMSRA

       490
QILQQAGTSV LAQANQVPQN VLSLLR
```

Figure 79

Flagellin from Listeria monocytogenes (accession number Q92DW3)

```
 *       10 *       *20          30     *     40          50      *    60
MKVNTNIISL KTQEYLRKNN EGMTQAQERL ASGKRINSSL DDAAGLAVVT RMNVKSTGLD

   *     70          80          90         100         *           *
AASKNSSMGI DLLQTADSAL SSMSSILQRM RQLAVQSSNG SFSDEDRKQY TAEFGSLIKE

       130     *    140         150     *   160        *170         180
LDHVADTTNY NNIKLLDQTA TNAATQVSIQ ASDKANDLIN IDLFNAKGLS AGTITLGSGS

       190         200         210         220         230          *
TVAGYSALSV ADADSSQEAT EAIDELINNI SNGRALLGAG MSRLSYNVSN VNNQSIATKA

       250          *  *       270         280
SASSIEDADM AAEMSEMTKY KILTQTSISM LSQANQTPQM LTQLINS
```

Figure 80