**Figure 1A: 95-2**

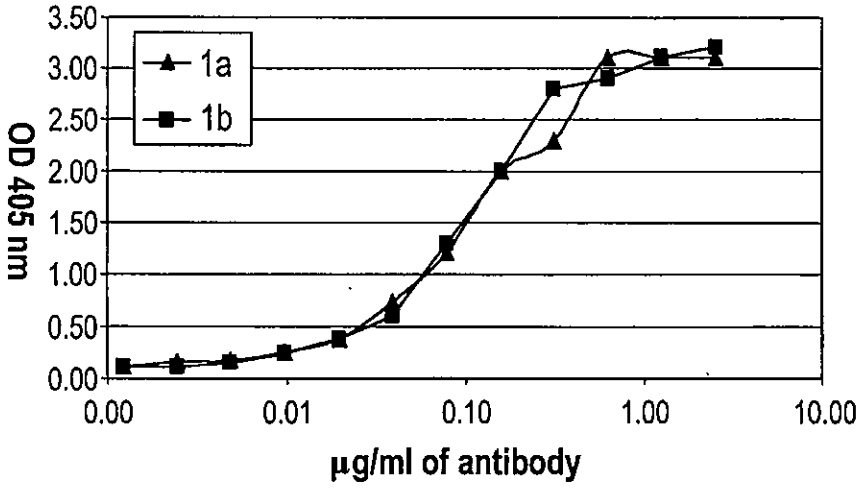
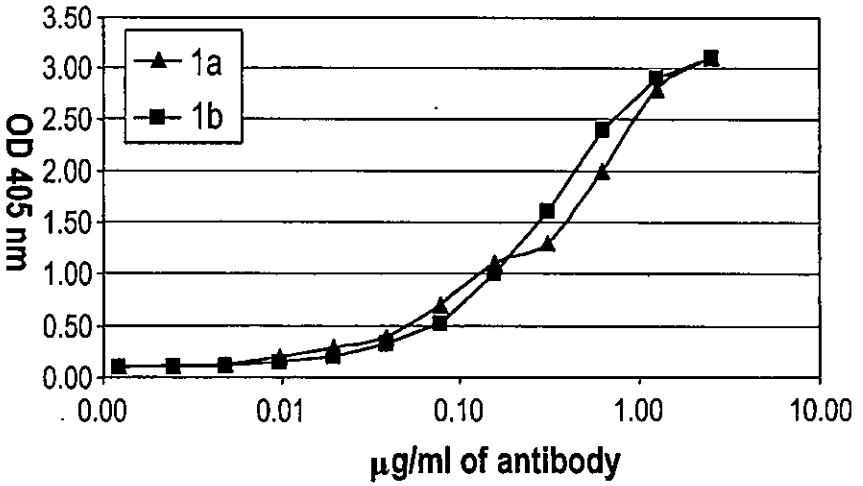
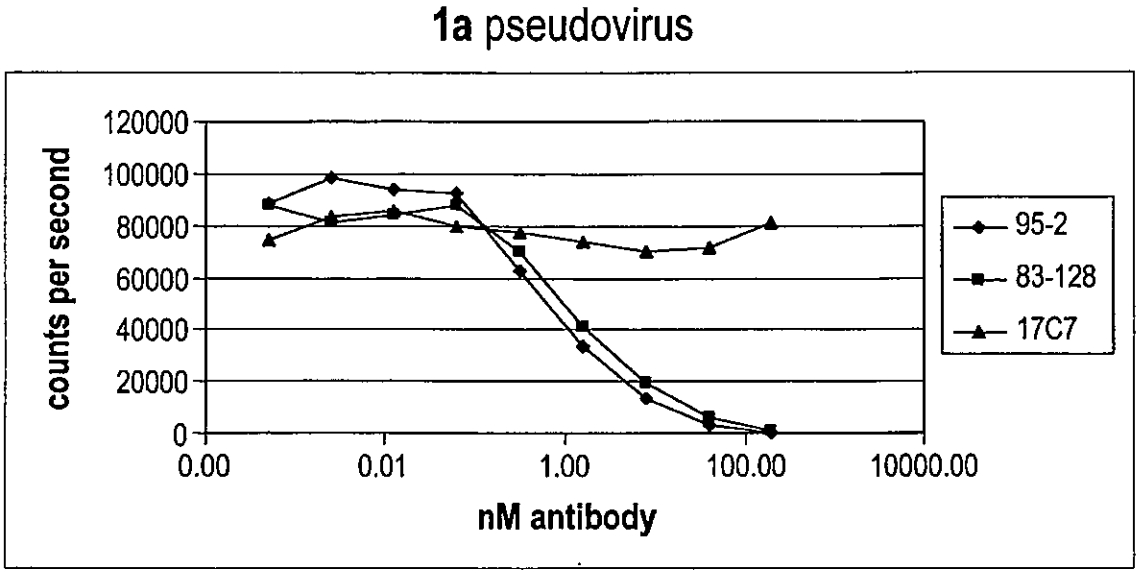


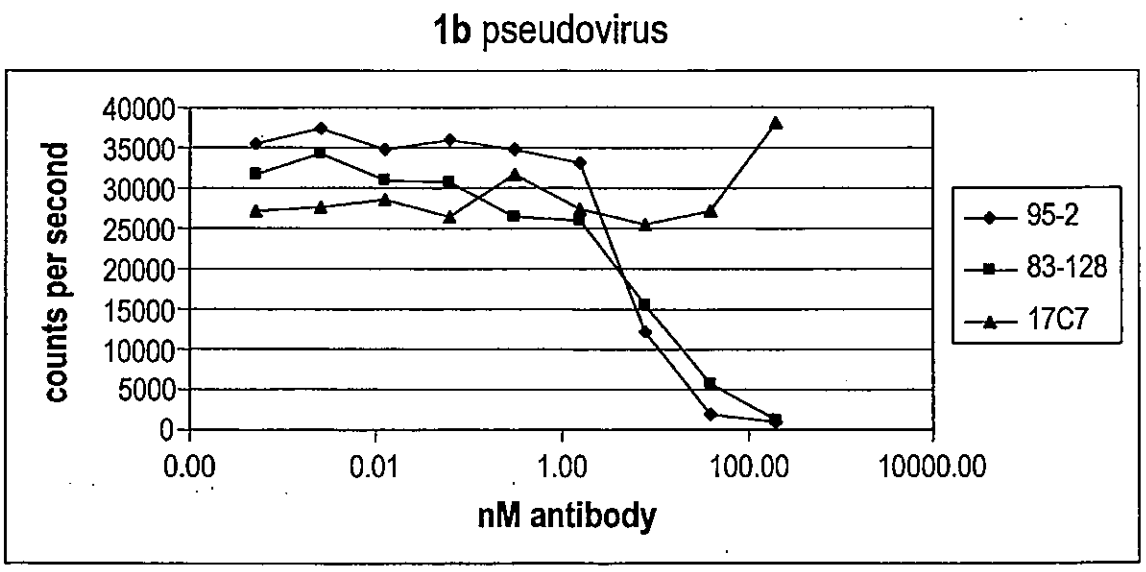

**Figure 1B: 83-128**




**Genotype 1 soluble E2 ELISA.** ELISA plates were coated with 2 µg/ml of 1a E2-660 (triangles) or 1b E2-661 (squares) and probed with HuMabs 95-2 and 83-128 in two-fold dilutions. Bound antibody was detected with goat-anti-human secondary antibody conjugated to alkaline phosphatase and PNPP substrate.

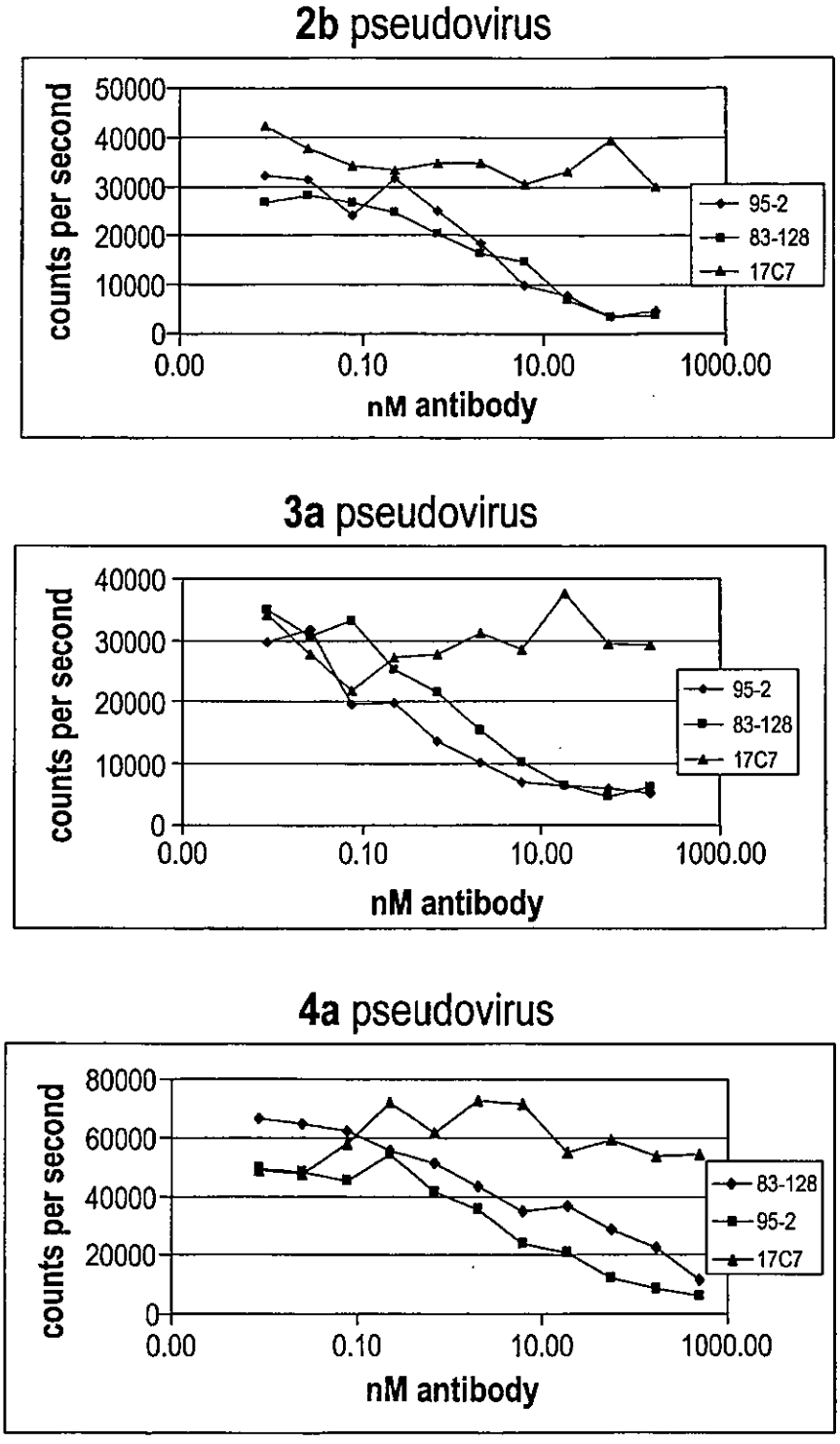
*Fig. 1*




**HCV genotype 1a and 1b pseudovirus neutralization.** Five-fold dilutions of HuMabs 95-2, 83-128, and 17C7 (rabies HuMab, negative control) were incubated with HCV pseudovirus for 1 hr at room temperature. The virus-antibody mixture was added to Hep3B cells followed by incubation at 37°C for 72 hrs. Infection was quantitated with Brightglo luciferase assay and read in a Victor3 plate reader for light output.

*Fig. 2A*




**HCV genotype 2b, 3a and 4a pseudovirus neutralization.** Five-fold dilutions of HuMabs 95-2, 83-128, and 17C7 (rabies HuMab, negative control) were incubated with HCV pseudovirus for 1 hr at room temperature. The virus-antibody mixture was added to Hep3B cells followed by incubation at 37°C for 72 hrs. Infection was quantitated with Brightglo luciferase assay and read in a Victor3 plate reader for light output.

*Fig. 2B*

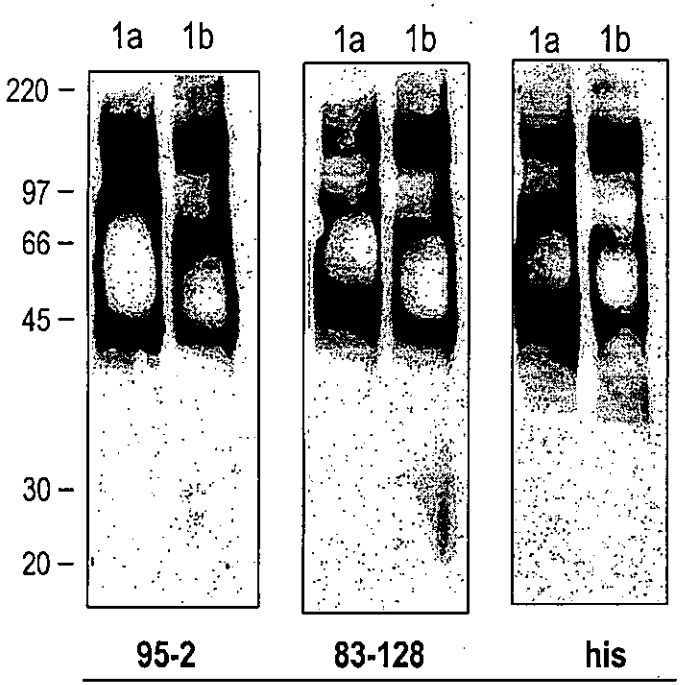



**Figure 3A: Reduced**

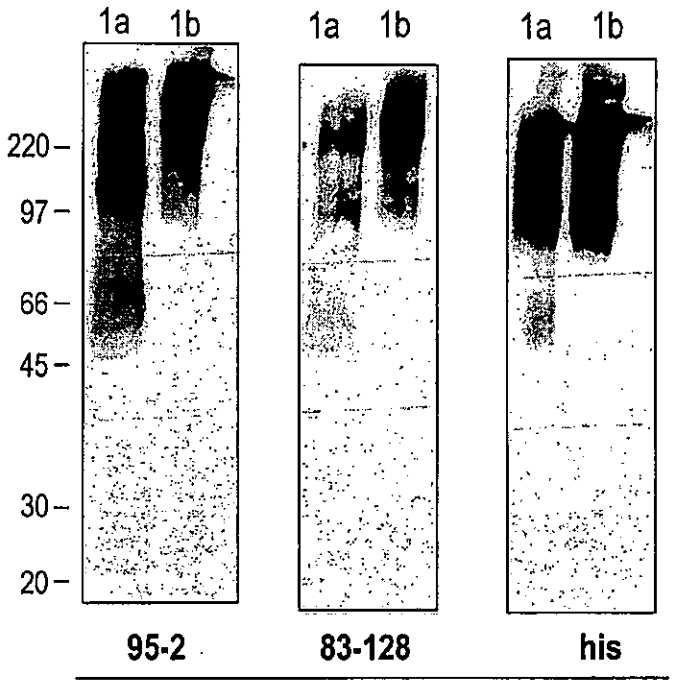



**Figure 3B: Non-Reduced**



**Reducing and non-reducing Western blots of genotype 1a and 1b E2 soluble protein.** Purified soluble mammalian expressed E2 protein (E2-661) from genotype 1a and 1b was subjected to reducing or non-reducing SDS-PAGE followed by transfer to PVDF membrane. Westerns blots were performed with anti-his tag monoclonal (his), 83-128 and 95-2 using anti-mouse IgG (his) or anti-human IgG (95-2 and 83-128) conjugated to HRP with enhanced chemiluminescent detection reagent. The genotype of the soluble E2 is indicated above the blots. The primary antibody used for detection is listed below the blots. The molecular weight markers in kilodaltons is listed to the left of each set of blots.

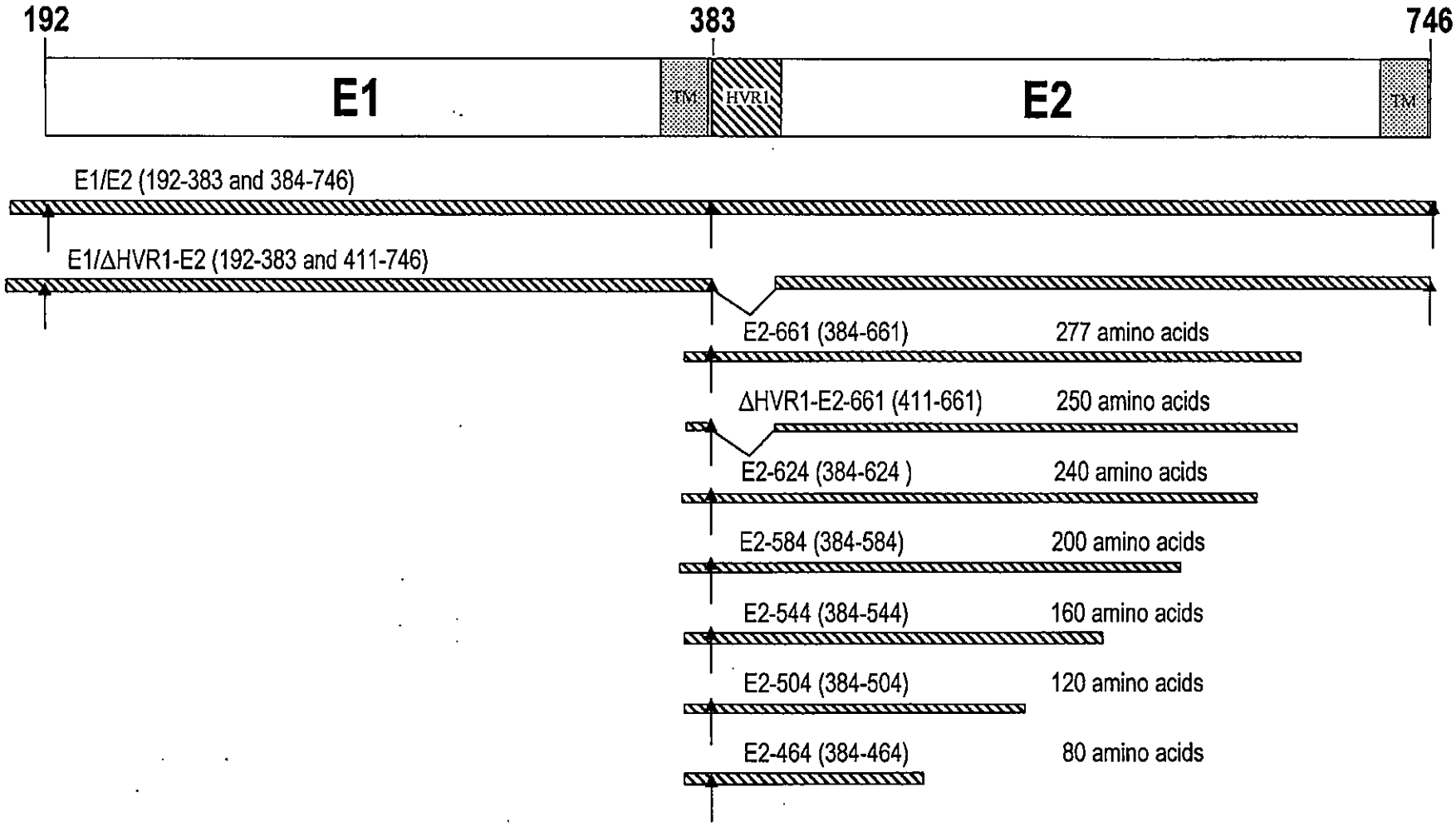
*Fig. 3*

| | ka (1/Ms) | kd (1/s) | KD (M) |
|---|---|---|---|
| 95-2 | 1.6e5 | 3.0e-4 | 1.9e-9 |
| 83-128 | 1.9e5 | 7.3e-4 | 3.8e-9 |



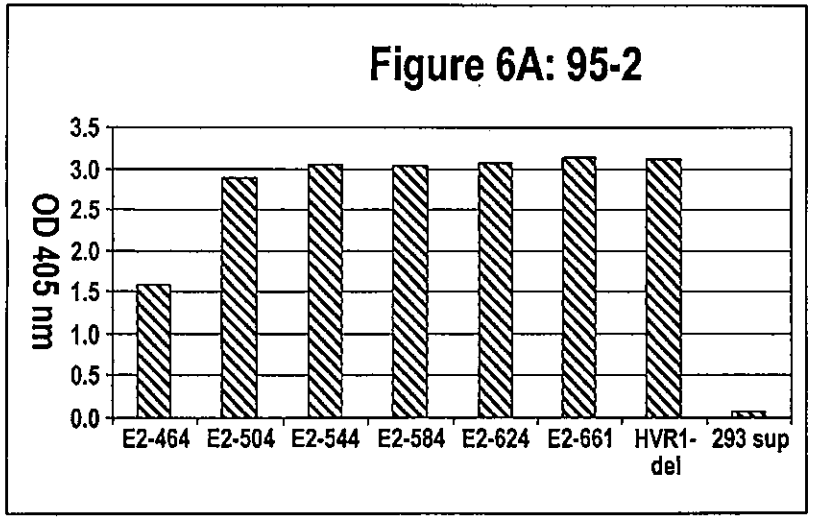
**95-2 and 83-128 affinity determination for E2 412-423 epitope expressed as a bacterial fusion protein.** Goat anti-human IgG Fc was amide coupled to the Biacore chip. 95-2 and 83-128 were separately captured on the chip and E2-G bacterially expressed protein containing E2 412-423 amino acids was flowed over at varying concentrations. The BIAevaluation software was used to fit the curves and calculate the affinity constants.

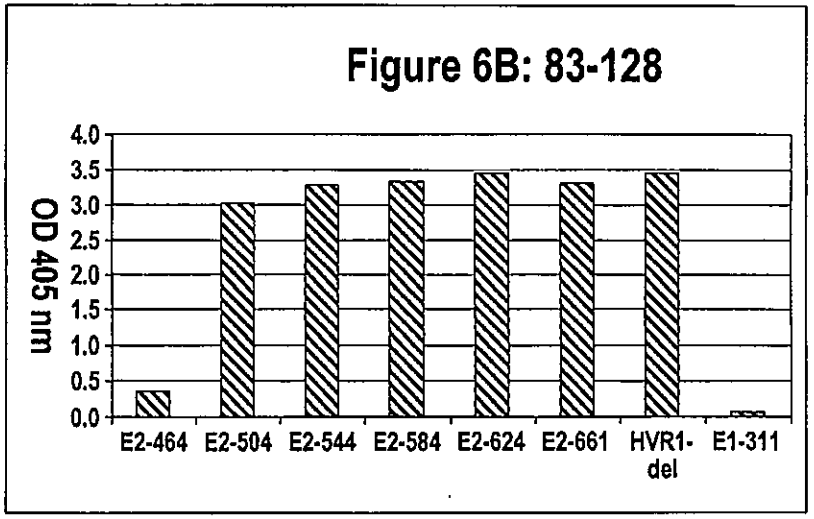
*Fig. 4*




**E1 and E2 mammalian expressed proteins.** The E1 and E2 polyprotein is indicated at top by a long white box with amino acid numbers noted above. The transmembrane domains (TM) are indicated by light grey boxes and the hypervariable domain 1 (HVR1) of E2 is indicated by a dark grey box. The mammalian proteins expressed from engineered constructs are listed below as dark grey bars. Sites of signal peptidase cleavage are indicated by arrows. The name of each protein is followed by the encoded amino acids in parenthesis and total amino acids in the expressed protein

*Fig. 5*

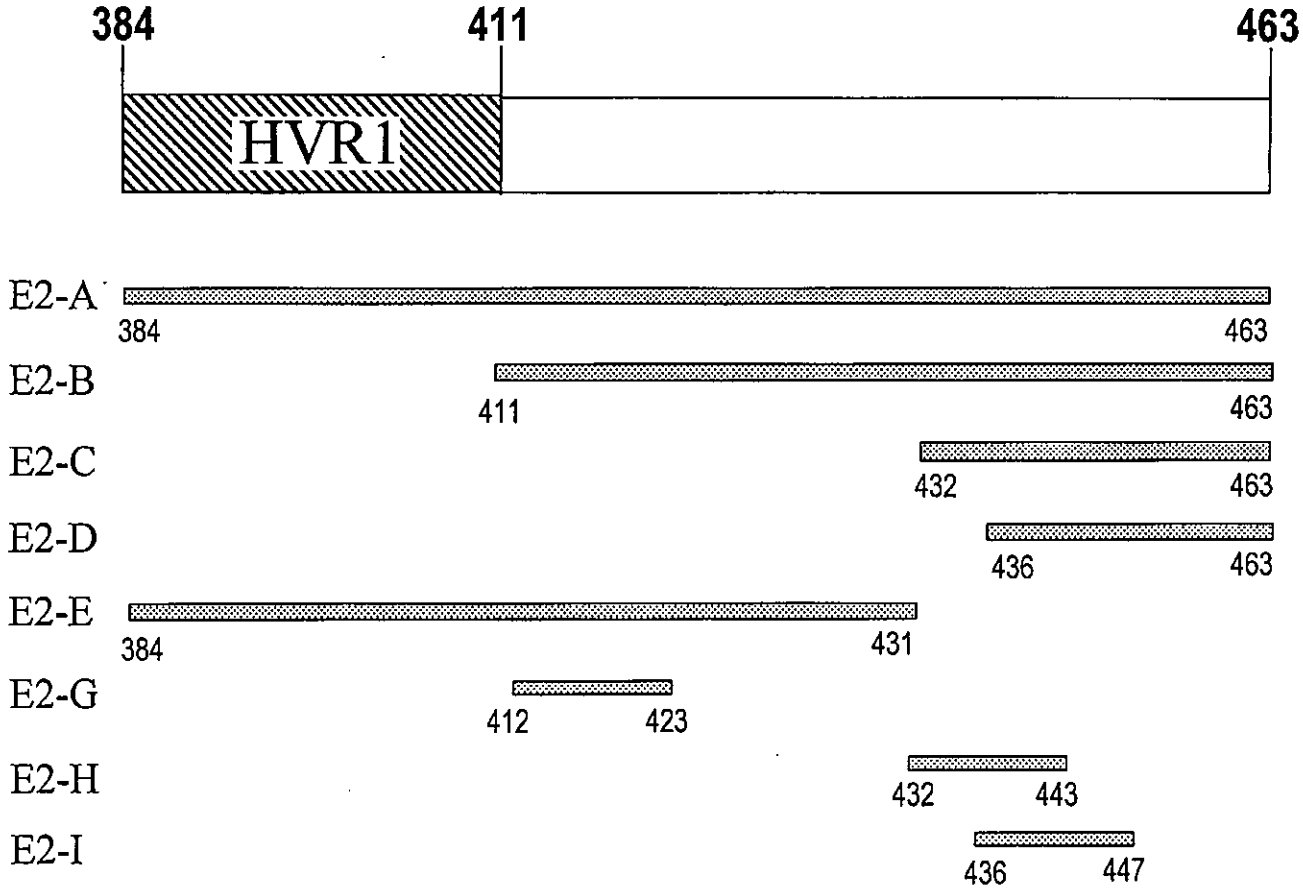





**Lectin capture ELISA of mammalian expressed C-terminal truncations of E2.** Cell culture supernatants containing mammalian expressed C-terminal truncations of E2, fusion proteins containing E2 amino acids indicated in figure 7 were coated on an ELISA plate and probed with two-fold dilutions of HuMabs 95-2 and 83-128 and mouse six histidine tag antibody (his tag). Bound antibodies were detected with goat-anti-human (95-2 and 83-128) or goat-anti-mouse (his tag) conjugated to alkaline phosphatase and PNPP substrate.

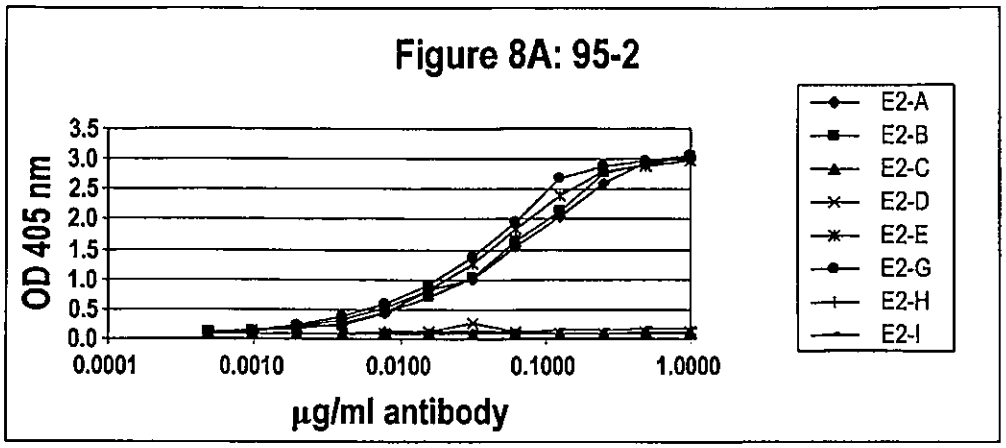
*Fig. 6*

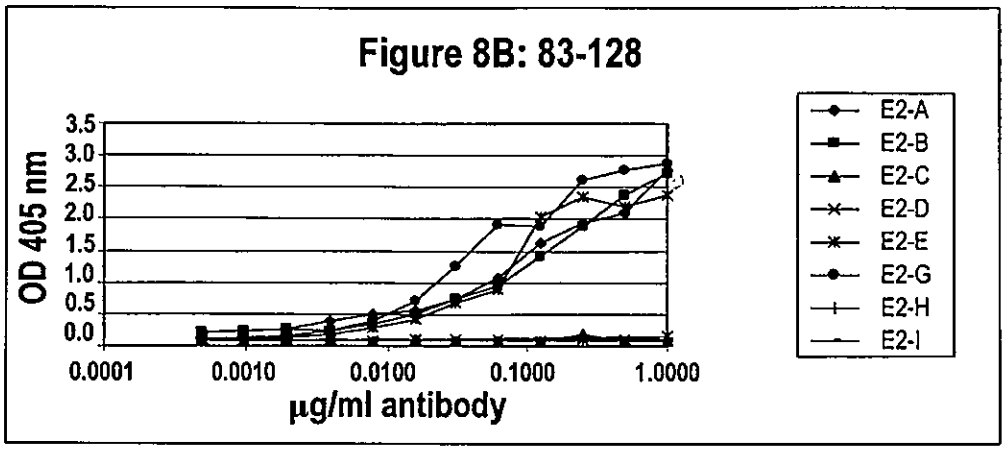


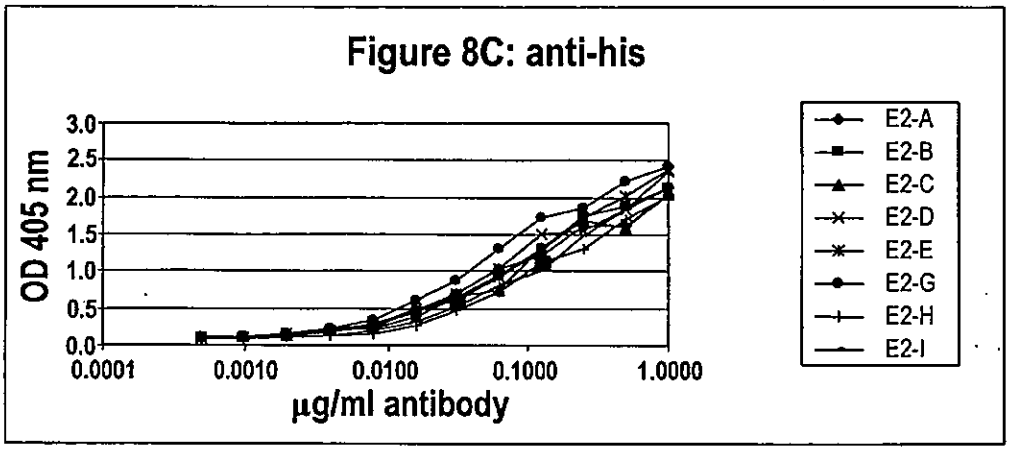



**Bacterially expressed proteins spanning the amino-terminal 80 amino acids of E2.** The first 80 amino acids of the E2 protein are represented at top by a white box with the amino acids numbers above. The 27 amino acid hypervariable region 1 (HVR1) at the amino-terminus is indicated by a dark grey box. The proteins expressed from engineered constructs are indicated by light grey bars with names to the left of the bar and amino acid numbers for the start and end of the protein listed below the bar.

*Fig. 7*

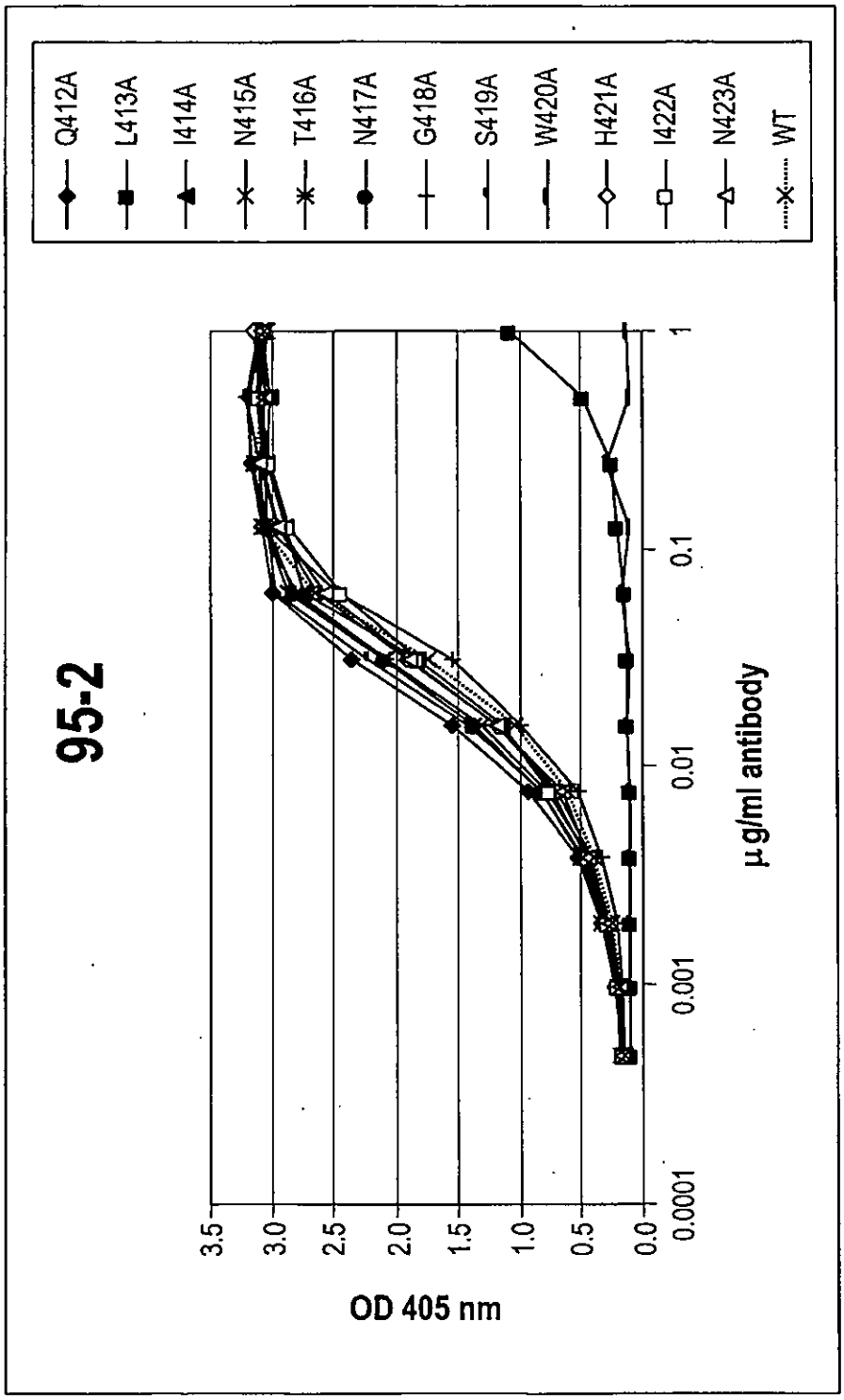


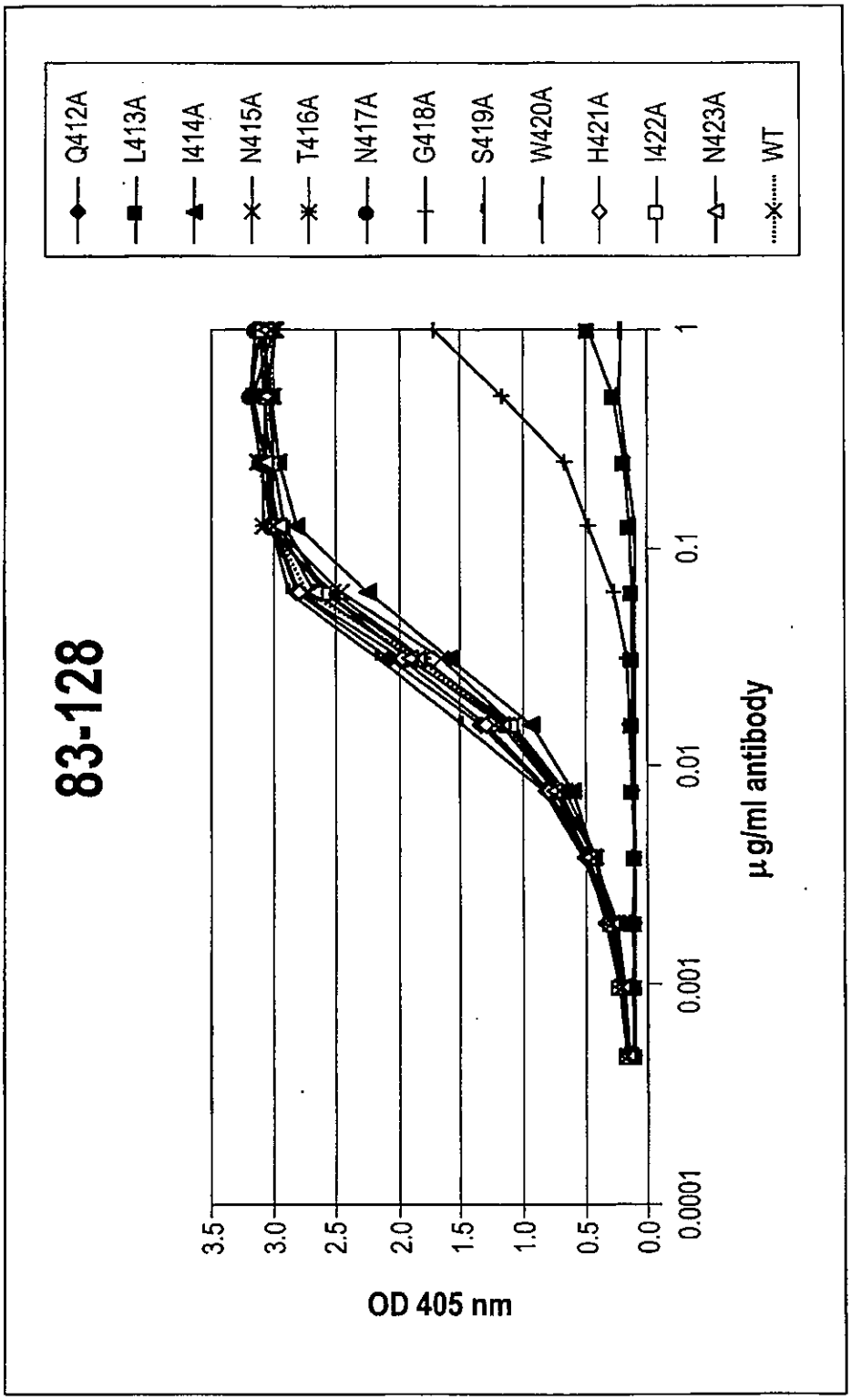

**ELISA of bacterially expressed pieces of E2 first 80 amino acids.** Bacterially expressed fusion proteins containing E2 amino acids indicated in figure 7 were coated on an ELISA plate and probed with two-fold dilutions of HuMabs 95-2 and 83-128 and mouse six histidine tag antibody (his tag). Bound antibodies were detected with goat-anti-human (95-2 and 83-128) or goat-anti-mouse (his tag) conjugated to alkaline phosphatase and PNPP substrate.

*Fig. 8*



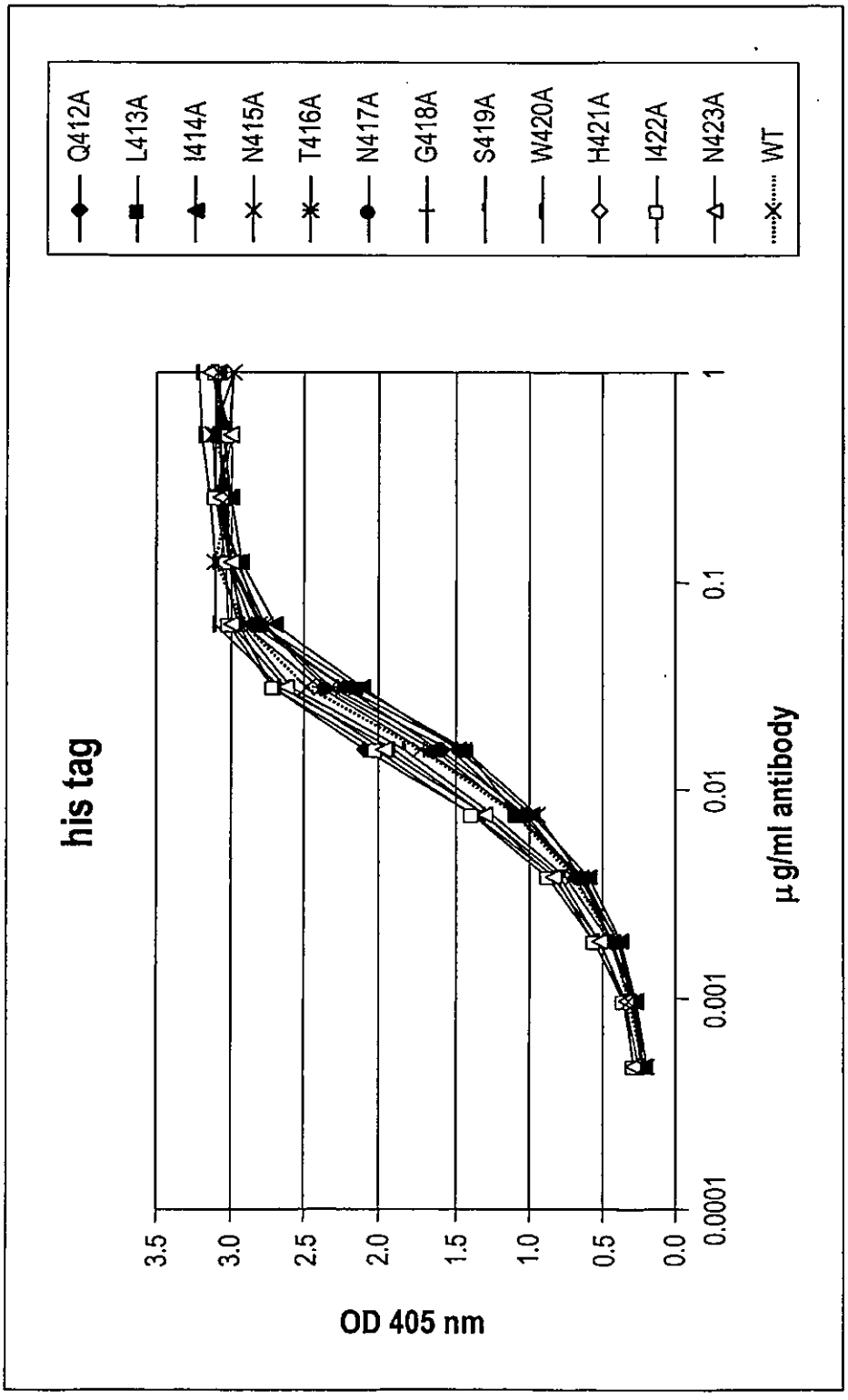

**ELISA of E2-412-423 alanine scanning mutants.** Bacterially expressed fusion proteins containing E2 amino acids 412-423 with the indicated amino acids mutated to an alanine or containing no mutantions (WT) were coated on an ELISA plate and probed with two-fold dilutions of HuMabs 95-2 and 83-128 and mouse six histidine tag antibody (his tag). Bound antibodies were detected with goat-anti-human (95-2 and 83-128) or goat-anti-mouse (his tag) conjugated to alkaline phosphatase and PNPP substrate.

*Fig. 9A*




**ELISA of E2-412-423 alanine scanning mutants.** Bacterially expressed fusion proteins containing E2 amino acids 412-423 with the indicated amino acids mutated to an alanine or containing no mutantions (WT) were coated on an ELISA plate and probed with two-fold dilutions of HuMabs 95-2 and 83-128 and mouse six histidine tag antibody (his tag). Bound antibodies were detected with goat-anti-human (95-2 and 83-128) or goat-anti-mouse (his tag) conjugated to alkaline phosphatase and PNPP substrate.

*Fig. 9B*




**ELISA of E2-412-423 alanine scanning mutants.** Bacterially expressed fusion proteins containing E2 amino acids 412-423 with the indicated amino acids mutated to an alanine or containing no mutantions (WT) were coated on an ELISA plate and probed with two-fold dilutions of HuMabs 95-2 and 83-128 and mouse six histidine tag antibody (his tag). Bound antibodies were detected with goat-anti-human (95-2 and 83-128) or goat-anti-mouse (his tag) conjugated to alkaline phosphatase and PNPP substrate.

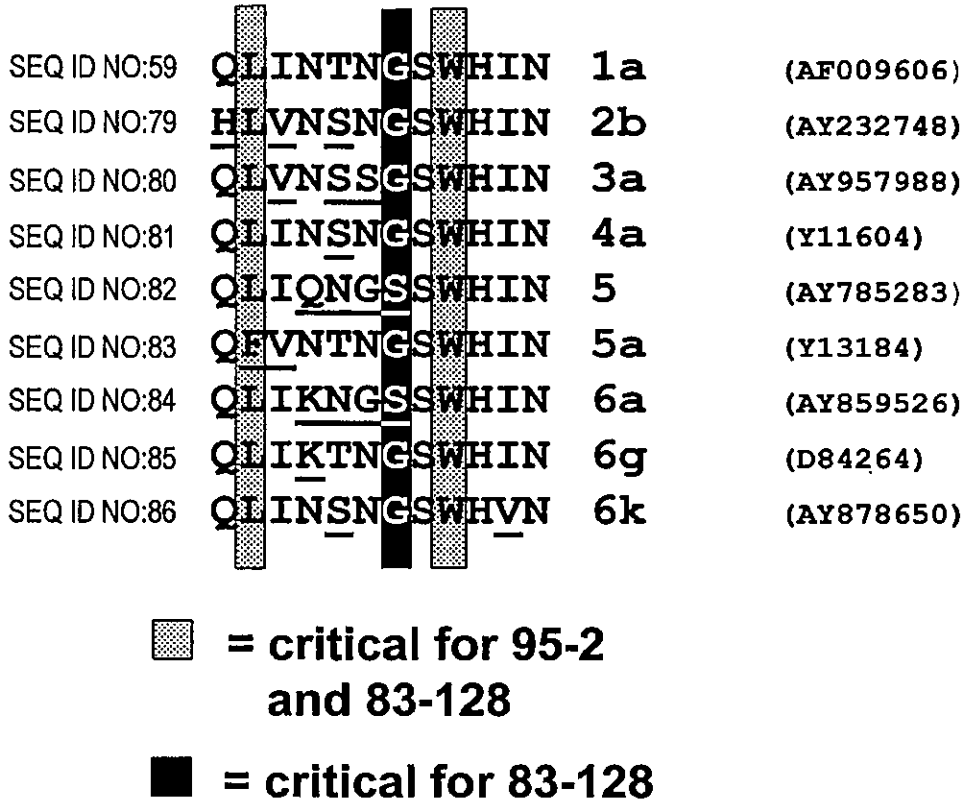
*Fig. 9C*

*Fig. 10*

| | | | Count | Percent |
|---|---|---|---|---|
| QLINTNGSWHIN | (1a) | (SEQ ID NO:59) | 78 | 48.1 |
| --V--------- | (1b) | (SEQ ID NO:60) | 48 | 29.6 |
| --V-S------- | | (SEQ ID NO:61) | 8 | 4.9 |
| ----S------- | | (SEQ ID NO:62) | 5 | 3.1 |
| H---------- | | (SEQ ID NO:63) | 4 | 2.5 |
| ---K-------- | | (SEQ ID NO:64) | 3 | 1.9 |
| --V-------V- | | (SEQ ID NO:65) | 1 | 0.6 |
| -FV--------- | | (SEQ ID NO:66) | 1 | 0.6 |
| ---KNGS----- | | (SEQ ID NO:67) | 1 | 0.6 |
| --VK-------- | | (SEQ ID NO:68) | 1 | 0.6 |
| H-V--------- | | (SEQ ID NO:69) | 1 | 0.6 |
| H-V-S------- | | (SEQ ID NO:70) | 1 | 0.6 |
| ---H-------- | | (SEQ ID NO:71) | 1 | 0.6 |
| --VK-E-N---- | | (SEQ ID NO:72) | 1 | 0.6 |
| N--K-------- | | (SEQ ID NO:73) | 1 | 0.6 |
| ---Y-------- | | (SEQ ID NO:74) | 1 | 0.6 |
| ----------L- | | (SEQ ID NO:75) | 1 | 0.6 |
| Y---------- | | (SEQ ID NO:76) | 1 | 0.6 |
| S---------- | | (SEQ ID NO:77) | 1 | 0.6 |
| N---------- | | (SEQ ID NO:78) | 1 | 0.6 |

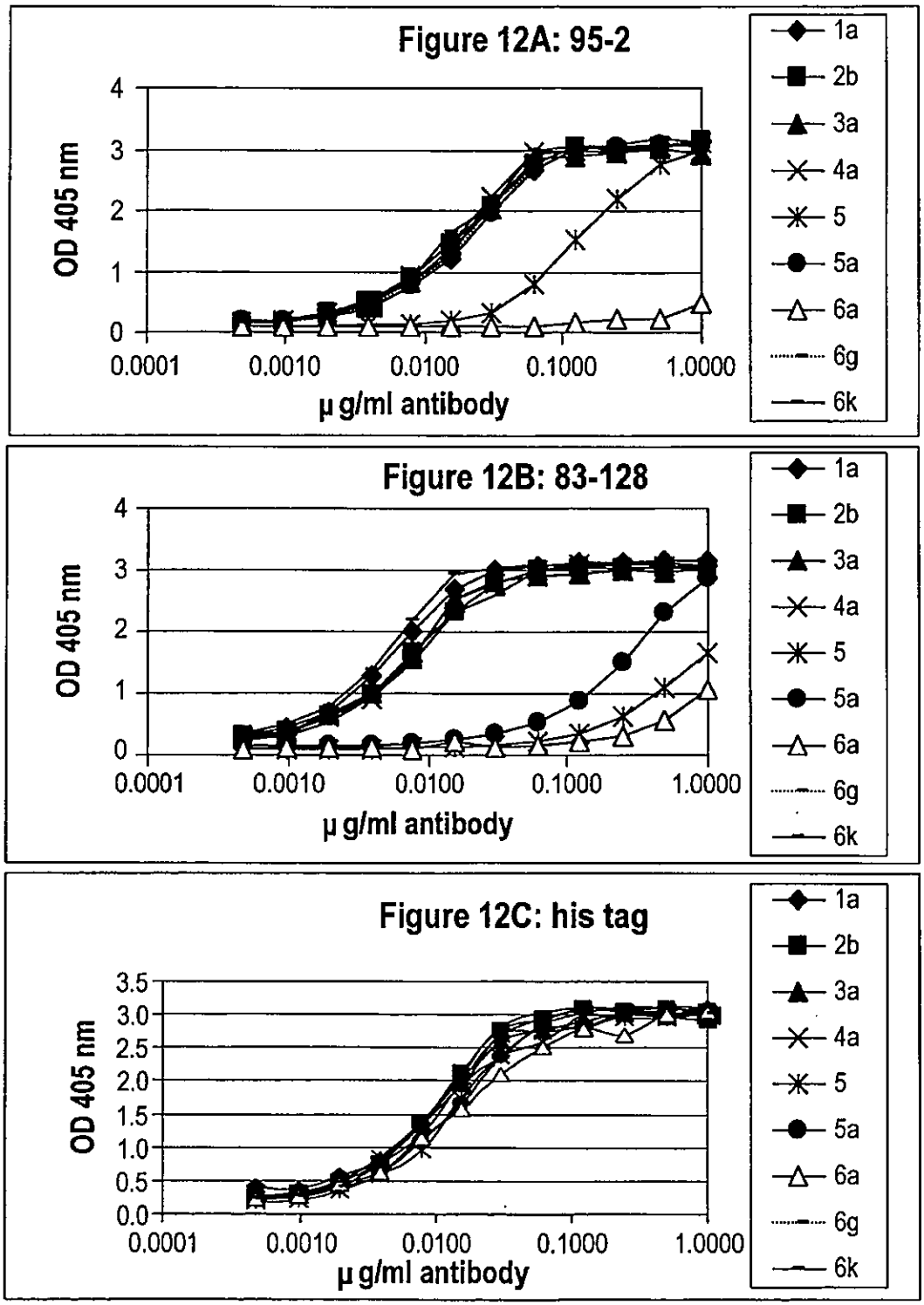
Total sequences = 160
Number of variants = 20

**E2 amino acids 412-423 alignments from the Los Alamos HCV Sequence Database supported by NIH.** The one letter amino acid code for the prototype genotype 1a sequence for amino acids 412-423 are listed at the top of chart in red (1a). Any amino acids that are identical to the 1a sequence are indicated with a dash and the amino acid listed for any position that is different from the prototype genotype 1a sequence. The number of times that this 12 amino acid sequence is found in the condensed database is listed under count and the percent of the total is listed under percent. The sequences of our 1a and 1b soluble E2 proteins are in red.




**E2 amino acids 412-423 from other genotypes.** The one-letter amino acid code for amino acids 412-423 are listed for isolates that contained changes (red underline) from the prototype genotype 1a sequence. The genotype name is listed to the right followed by the genbank accession number in parenthesis.

*Fig. 11*




**ELISA of E2 amino acids 412-423 from other genotypes.** Bacterially expressed fusion proteins containing E2 amino acids 412-423 with the sequence from the indicated genotype were coated on an ELISA plate and probed with two-fold dilutions of HuMabs 95-2 and 83-128 and mouse six histidine tag antibody (his tag). Bound antibodies were detected with goat-anti-human (95-2 and 83-128) or goat-anti-mouse (his tag) conjugated to alkaline phosphatase and PNPP substrate.

*Fig. 12*

E1/E2 codon optimized 1a H77 sequence with added ends containing restrictions sites (bold lowercase), additional overhangs to insure restriction enzyme cutting (underlined lowercase), and consensus Kozak sequence (bold underlined lowercase) with ATG initiation codon (bold underlined uppercase) (SEQ ID NO:31)

cgctatag**aagcttgccgccacc**ATGGCCACCGGCAACCTGCCCGGCTGCTCCTTC
TCCATCTTCCTGCTGGCCCTGCTGTCCTGCCTGACCGTGCCCGCCTCCGCCTACCAGGTGC
GCAACTCCTCCGGCCTGTACCACGTGACCAACGACTGCCCTAACTCCTCCATCGTGTACGA
GGCCGCCGACGCCATCCTGCACACTCCTGGCTGCGTGCCCTGCGTGCGCGAGGGCAACGCC
TCCCGCTGCTGGGTGGCCGTGACTCCTACCGTGGCCACCCGCGACGGCAAGCTGCCCACCA
CCCAGCTGCGCCGCCACATCGACCTGCTGGTGGGCTCCGCCACCCTGTGCTCCGCCCTGTA
CGTGGGCGACCTGTGCGGCTCCGTGTTCCTGGTGGGCCAGCTGTTCACCTTCTCTCCTCGC
CGCCACTGGACCACCCAGGACTGCAACTGCTCCATCTACCCTGGCCACATCACCGGCCACC
GCATGGCCTGGGACATGATGATGAACTGGTCTCCCACCGCCGCCCTGGTGGTGGCCCAGCT
GCTGCGCATCCCTCAGGCCATCATGGACATGATCGCCGGCGCCCACTGGGGCGTCCTGGCC
GGCATCGCCTACTTCTCCATGGTGGGCAACTGGGCCAAGGTGCTGGTGGTGCTGCTGCTGT
TCGCCGGCGTGGACGCCGAGACCCACGTGACGGGCGGCTCCGCCGGCCGCACCACCGCCGG
CCTGGTGGGCCTGCTGACACCCGGCGCCAAGCAGAACATCCAGCTGATCAACACCAACGGC
TCCTGGCACATCAACTCCACCGCCCTGAACTGCAACGAGTCCCTGAACACCGGCTGGCTGG
CCGGCCTGTTCTACCAGCACAAGTTCAACTCCTCCGGCTGTCCCGAGCGCCTGGCCTCCTG
CCGCCGCCTGACCGACTTCGCCCAGGGCTGGGGCCCTATCTCCTACGCCAACGGCTCCGGC
CTGGACGAGCGGCCCTACTGCTGGCACTACCCTCCTCGCCCTTGCGGCATCGTGCCCGCCA
AGTCCGTGTGCGGCCCTGTGTACTGCTTCACTCCCTCTCCCGTGGTGGTGGGCACCACCGA
CCGCTCCGGCGCTCCCACCTACTCCTGGGGCGCCAACGACACCGACGTGTTCGTGCTGAAC
AACACCCGCCCTCCTCTGGGCAACTGGTTCGGCTGCACCTGGATGAACTCCACCGGCTTCA
CCAAGGTGTGCGGCGCGCCTCCCTGCGTGATCGGCGGCGTGGGCAACAACACCCTGCTGTG
CCCTACCGACTGCTTCCGCAAGCATCCCGAGGCCACCTACTCCCGCTGCGGCTCCGGGCCC
TGGATCACTCCTCGCTGCATGGTGGACTATCCCTACCGCCTGTGGCACTATCCCTGCACCA
TCAACTACACCATCTTCAAGGTGCGCATGTACGTGGGCGGCGTGGAGCACCGCCTGGAGGC
GGCGTGCAACTGGACCCGCGGCGAGCGCTGCGACCTGGAGGACCGCGACCGCTCCGAGCTG
TCTCCTCTGCTGCTGTCCACCACCCAGTGGCAGGTGCTGCCCTGCTCCTTCACCACCCTGC
CCGCGCTGTCCACCGGCCTGATCCACCTGCACCAGAACATCGTGGACGTGCAGTACCTGTA
CGGCGTGGGCTCCTCCATCGCGTCCTGGGCGATCAAGTGGGAGTACGTGGTGCTGCTGTTC
CTGCTGCTGGCGGACGCGCGCGTGTGCTCCTGCCTGTGGATGATGCTGCTGATCTCCCAGG
CGGAGGCG**tctaga**ggagtca

*Fig. 13*

ALIGNMENT AND CONSENSUS SEQUENCE FOR MONOCLONAL ANTIBODIES REACTIVE AGAINST EPITOPE 412-423 OF E2-660 HCV GLYCOPROTEIN Germ line: VH3-33, D3-10, JH4; Vk-L6, Jk5

**Heavy Chain Amino Acid Alignment**

```
95-43 (SEQ ID NO:36)    QVQLVESGGGVVQPGRSLRLSCAASGFTFSSYGMHWVRQAPGKGLEWVAVIWFDGNNKYY
95-54  (38)             QVQLVESGGGVVQPGRSLRLSCAASGFTFSSYGMHWVRQAPGKGLEWVAVIWFDGNNKYY
95-25  (34)             QVQLVESGGGVVQPGRSLRLSCAASGFTFSNYGMHWVRQAPGKGLEWVAVIWFDGNNKYY
95-14  (32)             QVQLVESGGGVVQPGRSLRLSCAASGFILSSYGMHWVRQAPGKGLEWVAVIWYDGNNKYY
95-49  (37)             QVQLVESGGGVVQPGRSLRLSCAASGFTLSSYGMHWVRQAPGKGLEWVAVIWYDGNNKYY
95-62  (40)             QVQLVESGGGVVQPGRSLRLSCAASGFTLSSYGMHWVRQAPGKGLEWMAVIWYDGNNKYY
95-42  (35)             QVQLVESGGGVVQPGRSLRLSCAASGFTFSSYGMHWVRQAPGKGLEWVAVIWYDESNKYY
95-58  (39)             QVQLVESGGGVVQPGRSLRLSCAASGFTFSSYGMHWVRQAPGKGLEWVAVIWYDENNKYY
95-2   (3)              QVQLVESGGGVVQPGRSLRLSCAASGFTFSSYGMHWVRQAPGKGLEWVAVIWFDGNNQYY
95-38  (33)             QVQLVESGGGVVQPGRSLRLSCAASGFTFSSYGMHWVRQAPGKGLEWVAVIWLDGSNTYY
83-128 (1)              QVQLVESGGGVVQPGRSLRLSCTASGFTFNNYGMHWVRQTPGKGLEWLAVIWFDENNKYY
                        ************************:******:..********:*******:**** * .* **

95-43                   ADSMKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARDIFTMVRGVFIYFDYWGQGT
95-54                   ADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARDIFTMVRGVFIYFDYWGQGT
95-25                   ADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARDIFTMDRGVFIYFDYWGQGT
95-14                   ADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARDIFTMVRGVFIYFDYWGQGT
95-49                   ADSVKGRFTISRDNSKNTLYLQMDSLRAEDTAVYYCARDIFTMVRGVFIYFDYWGQGT
95-62                   ADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARDIFTMVRGVFIYFDYWGQGT
95-42                   ADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARDIFTMVRGVFIYFDYWGQGT
95-58                   ADSVKGRFTISRDNSNNTLYLQMNSLRAEDTAVYYCARDIFTMVRGVFIYFDYWGQGT
95-2                    ADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARDIFTVVRGFFIYFDYWGQGT
95-38                   ADSVKGRFTISRDNSKNTLFLQMNSLRAEDTAVYYCARDIFTVARGVIIYFDYWGQGT
83-128                  ADSVRGRFTISRDNSKNTLFLQMNSLKTEDTAMYYCARDI-SLVRDAFIYFDFWGLGT
                        ***::**********************::****:******* ::.*..:****:** **

95-43                   LVTVSS
95-54                   LVTVSS
95-25                   LVTVSS
95-14                   LVTVSS
95-49                   LVTVSS
95-62                   LVTVSS
95-42                   LVTVSS
95-58                   LVTVSS
95-2                    LVTVSS
95-38                   LVTVSS
83-128                  LVTVSS
                        ******
```

*Fig. 14*

**Light Chain Amino Acid Alignments**

```
073-1   (SEQ ID NO: 6)     EIVLTQSPATLSLSPGERATLSCRASQSVSSYLAWYQQKPGQAPRLLIYDASNRATGIPA
95-38   (53)               EIVLTQSPATLSLSPGERATLSCRASQSVSSYLAWYQQKPGQAPRLLIYDASNRATGIPA
95-2    (4)                EIVLTQSPATLSLSPGERATLSCRASQSVGSYLAWYQQKPGQAPRLLIYDASNRATGIPA
95-14   (44)               EIVLTQSPATLSLSPGERATLSCRASQSVGSYLAWYQQKPGQAPRLLIYDASNRATGIPA
83-128  (2)                EIVLTQSPATLSLSPGERATLSCRASQSVSSYLAWYQQKPGQAPRLLIYDASNRATGIPA
                           ******************************.******************************

073-1                      RFSGSGSGTDFTLTISTLEPEDFAVYYCQQRSNWVTFGQGTRLEIK
95-38                      RFSGSGSGTDFTLTISSLEPEDFAVYYCQQRSNWVTFGQGTRLEIK
95-2                       RFSGSGSGTDFTLTISSLEPEDFAVYYCQQRSNWVTFGQGTRLEIK
95-14                      RFSGSGSGTDFTLTISSLEPEDFAVYYCQQRSNWVTFGQGTRLEIK
83-128                     RFSGSGSGTDFTLTISSLEPEDFAVYYCQQRSNWITFGQGTRLEIK
                           ****************:*****************:***********
```

*Fig. 15*