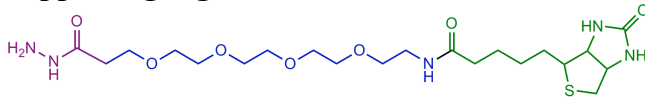


Supporting information

Chemical labelling of active serum thioester proteins for quantification.

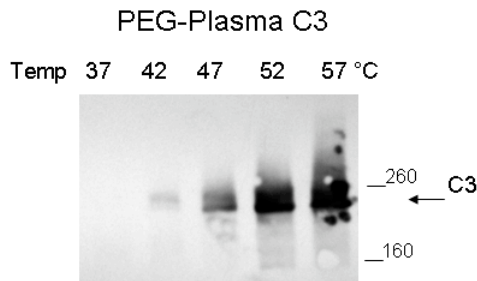
Lotta Holm, Gareth L. Ackland, Mark R. Edwards, Ross A. Breckenridge, Robert B. Sim and John Offer.

Supporting Figure 1:

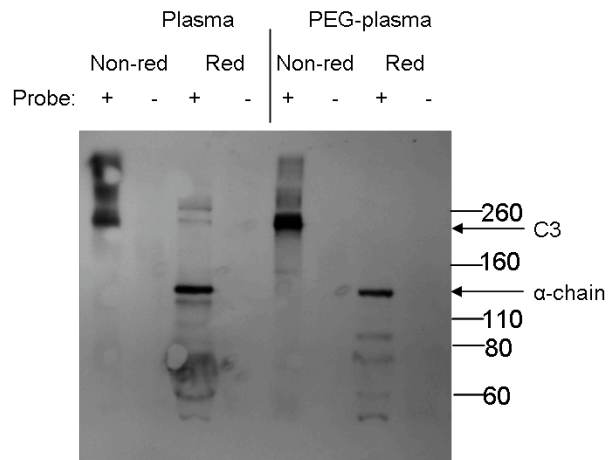


Chemical structure of thioester reactive probe: biotin-dPEG₄-hydrazide (Celares)

Supporting Figure 2:



Supporting Figure 2:



Supporting Material and Methods:

Peptide Mass Fingerprinting

MALDI-MS raw spectra for mass fingerprinting were processed using BioTools (Bruker Daltonics, MA). Data were processed using the smoothing algorithm SavitzkyGolay, baseline subtractions by the TopHat algorithm and peaks were centred at 80% peak high

Excision iv)

It could not be conclusively established which of the particular C4 isoforms (C4A, C4B or both) were present in this donor due to their extremely high sequence identity.

There is an extremely high sequence similarity between the two C4 isoforms (CO4A_HUMAN vs CO4B_HUMAN) in humans. A blast search was performed to find the positions of isoform unique amino acids and further compared peptide containing a unique site were identified in the MALDI-TOF experiment. There were no peptides from CO4A_HUMAN found in a sequence unique area. There was only one unique peptide from CO4B_HUMAN found but would need LC-MS-MS to satisfactorily characterise and identify peptides.

Supporting Figure 3:

Peptide coverage of identified peptides for excision i-iv, vi.

Excision i)

Match to: **A2MG_HUMAN** Score: 207 Expect: **4e-17**

Alpha-2-macroglobulin OS=Homo sapiens GN=A2M PE=1 SV=3

Nominal mass (M_r): **164613**; Calculated pI value: **6.03**

NCBI BLAST search of [A2MG_HUMAN](#) against nr

Unformatted [sequence string](#) for pasting into other applications

Taxonomy: [Homo sapiens](#)

Fixed modifications: Carbamidomethyl (C)

Variable modifications: Oxidation (M)

Cleavage by Trypsin: cuts C-term side of KR unless next residue is P

Number of mass values searched: **100**

Number of mass values matched: **40**

Sequence Coverage: **38%**

Matched peptides shown in **Bold Red**

```
1  MGKNKLLHPS  LVLLLLVLLP  TDASVSGKPQ  YMVLVPSLLH  TETTEKGCVL
51  LSYLNETVTV  SASLESVRGN  RSLFTDLEAE  NDVLHCVAFA  VPKSSSNEEV
101 MFLTQVKGK  TQEFKKRTTV  MVKNEDSLVF  VQTDKSIYKP  GQTVKFRVVS
151 MDENFHPLNE  LIPLVYIQDP  KGNRIAQWQS  FQLEGGLKQF  SFPLSSEPFQ
201 GSYKVVVQKK  SGGRTEHPFT  VEEFVLPKFE  VQVTVPKIIT  ILEEEMNVSV
251  CGLYTYGKPV  PGHVTVSICR  KYSDASDCHG  EDSQAFCEKF  SGQLNSHGCF
301 YQQVTKVFQ  LKRKEYEMKL  HTEAQIQEEG  TVVELTGRQS  SEITRTITKL
351  SFVKVDSHFR  QGIPFFGQVR  LVDGKGVPIP  NKVIFIRGNE  ANYYSNATTD
401  EHGLVQFSIN  TTNVMGTSLT  VRVNYKDRSP  CYGYQWVSEE  HEEAHTAYL
451 VFSPSKSFVH  LEPMSHELPC  GHTQTVQAHY  ILNGGTLGLL  KKLSFYLLIM
501 AKGGIVRTGT  HGLLVKQEDM  KGHFSISIPV  KSDIAPVARL  LIYAVLPTGD
551 VIGDSAKYDV  ENCLANKVDL  SFSPSQSLPA  SHAHLRVTAA  PQSVCALRAV
601  DQSVLLMKPD  AELSASSVYN  LLPEKDLTGF  PGPLNDQDNE  DCINRHNVII
651 NGITYTPVSS  TNEKDMYSFL  EDMGLKAFTN  SKIRKPKMCP  QLQQYEMHGP
701  EGLRVGFYES  DVMGRGHARL  VHVEEPHTET  VRKYFPETWI  WDLVVVNSAG
```

751 VAEVGVTPVD TIT~~EWK~~**AGAF** **CL**SEDA**GLGI** **SSTASL**RAFQ **PF**FVELT**MPY**
 801 **SVIR**GEAFTL KATVNLN**YLPK** CIR**VSVQ**LEA **SPAFLAVP**VE **KE**QAPHCICA
 851 NGRQTVSWAV TPKSLGNVNF TVSAEALESQ ELCGTEVPSV PEHGRK**DTVI**
 901 **KPLLVE**PEGL EK**ETTF**N**SLL** CP**SGGEV**SEE LSLK**LPPNVV** EESARASVSV
 951 LGDILGSAMQ NTQNL**LQMPY** GCGEQNMVLF APNIYVLDYL NETQQLT**PEI**
 1001 **KSKAIG**YLN**T** GYQRQLNYKH YDGS**YSTFGE** RYGR**NQGN**TW **LTA**FVL**KTF**A
 1051 QARAYIFIDE AHITQAL**IWL** SQRQK**DNGCF** R**SSG**SLL**NNA** IKGGVEDEVT
 1101 LSAYIT**IALL** EIPLTVTHPV VR**NALF**C**LES** **AWK**TAQEGDH GSHVY**T**K**ALL**
 1151 **AYAFAL**AGNQ **DKR**KEVLKSL NEEAVK**KDNS** **VH**WERP**QKPK** APVGHFYEPQ
 1201 APSAEVEMTS YVLLAYLTAQ PAPT**SED**LTS ATNIVK**WITK** **QQNA**QGG**FSS**
 1251 **TQDTV**VALHA **LSKY**GAATFT RT**GKAAQ**VTI **QSSG**TFSS**KF** QVDNN**N**RL**LL**
 1301 **QQVSL**PELPG EYS**MK**VTGEG CVYLQ**T**SLKY **NIL**PEKE**EF**P **FAL**GVQ**T**LPQ
 1351 **TCDEP**KAHTS **FQIS**LSVSYT **GSR**SAN**MAI** **VD**VKM**VSG**FI **PL**KPT**V**K**M**LE
 1401 RSNHVS**RTEV** SS**NH**VLIYLD **KVS**NQ**TLSL**F **FT**VLQ**D**VP**V**R DLKPA**I**V**K**VY
 1451 **DYYET**DEF**AI** **AEYN**AP**CSK**D LGNA

Excision ii)

Match to: **A2MG_HUMAN** Score: 71 Expect: 0.0016

Alpha-2-macroglobulin OS=Homo sapiens GN=A2M PE=1 SV=3

Nominal mass (M_r): 164613; Calculated pI value: 6.03

NCBI BLAST search of [A2MG_HUMAN](#) against nr

Unformatted [sequence string](#) for pasting into other applications

Taxonomy: [Homo sapiens](#)

Fixed modifications: Carbamidomethyl (C)

Variable modifications: Oxidation (M)

Cleavage by Trypsin: cuts C-term side of KR unless next residue is P

Number of mass values searched: 100

Number of mass values matched: 20

Sequence Coverage: 24%

Matched peptides shown in **Bold Red**

1 MGK**N**KL**LHPS** LV**LL**LLV**LLP** TDASVSG**KPQ** Y**M**VLVPS**LLH** TETTEK**GC**VL
 51 LS**Y**LN**ET**VT**V** SASLESV**RGN** **RSL**F**T**D**L**E**A**E **ND**VL**H**C**V**A**F**A **V**P**K**SS**S**N**E**E**V**
 101 M**F**LT**V**Q**V**K**GP** T**Q**E**F**K**R**RT**V** M**V**K**N**E**D**S**L**V**F** V**Q**T**D**K**S**I**Y**K**P** G**Q**T**V**K**F**R**V**V**S**
 151 **M**D**E**N**F**H**P**L**N**E **L**I**P**L**V**Y**I**Q**D**P **K**G**N**R**I**A**Q**W**Q**S **F**Q**L**E**G**G**L**K**Q**F **S**F**P**L**S**S**E**P**F**Q
 201 G**S**Y**K**V**V**V**Q**K **S**G**G**R**T**E**H**P**F**T **V**E**E**F**V**L**P**K**F**E **V**Q**V**T**V**P**K**I**I**T **I**L**E**E**E**M**N**V**S**V
 251 C**G**L**Y**T**Y**G**K**P**V** P**G**H**V**T**V**S**I**C**R** K**Y**S**D**A**S**D**C**H**G** E**D**S**Q**A**F**C**E**K**F** **S**G**Q**L**N**S**H**G**C**F
 301 **Y**Q**Q**V**K**T**K**V**F**Q **L**K**R**K**E**Y**E**M**K**L **H**T**E**A**Q**I**Q**E**E**G **T**V**V**E**L**T**G**R**Q**S **S**E**I**T**R**I**T**I**T**K**L**
 351 **S**F**V**K**V**D**S**H**R** **Q**G**I**P**F**F**G**Q**V**R **L**V**D**G**K**G**V**P**I**P **N**K**V**I**F**I**R**G**N**E **A**N**Y**S**N**A**T**T**D**
 401 E**H**G**L**V**Q**F**S**I**N** **T**T**N**V**M**G**T**S**L**T **V**R**V**N**Y**K**D**R**S**P **C**Y**G**Y**Q**W**S**E**E** **H**E**E**A**H**H**T**A**Y**L
 451 **V**F**S**P**S**K**S**F**V**H **L**E**P**S**M**S**H**E**L**P**C** **G**H**T**Q**T**V**Q**A**H**Y **I**L**N**G**G**T**L**L**G**L **K**K**L**S**F**Y**L**I**M**
 501 A**K**G**G**I**V**R**T**G**T** **H**G**L**L**V**K**Q**E**D**M **K**G**H**F**S**I**S**I**P**V **K**S**D**I**A**P**V**A**R**L **L**I**Y**A**V**L**P**T**G**D
 551 **V**I**G**D**S**A**K****Y**D**V** **E**N**C**L**A**N**K**V**D**L **S**F**S**P**S**Q**S**L**P**A **S**H**A**H**L**R**V**T**A**A **P**Q**S**V**C**A**L**R**A**V
 601 D**Q**S**V**L**L**M**K**P**D** A**E**L**S**A**S**S**V**Y**N** **L**L**P**E**K**D**L**T**G**F **P**G**P**L**N**D**Q**D**N**E **D**C**I**N**R**H**N**V**Y**I
 651 N**G**I**T**Y**T**P**V**S**S** **T**N**E**K**D**M**S**F**L** **E**D**M**G**L**K**A**F**T**N **S**K**I**R**K**P**K****M**C**P** **Q**L**Q**Y**E**M**H**G**P**
 701 **E**G**L**R**V**G**F**Y**S** **D**V**M**G**R**G**H**A**R**L **V**H**V**E**P**H**T**E**T** **V**R**K**Y**F**P**E**T**W**I **W**D**L**V**V**V**N**S**A**G
 751 VAEVGVTPVD TIT~~EWK~~**AGAF** **CL**SEDA**GLGI** **SSTASL**RAFQ **PF**FVELT**MPY**
 801 **SVIR**GEAFTL KATVNLN**YLPK** CIR**VSVQ**LEA **SPAFLAVP**VE **KE**QAPHCICA
 851 NGRQTVSWAV TPKSLGNVNF TVSAEALESQ ELCGTEVPSV PEHGRK**DTVI**
 901 **KPLLVE**PEGL EK**ETTF**N**SLL** CP**SGGEV**SEE LSLK**LPPNVV** EESARASVSV
 951 LGDILGSAMQ NTQNL**LQMPY** GCGEQNMVLF APNIYVLDYL NETQQLT**PEI**
 1001 **KSKAIG**YLN**T** GYQRQLNYKH YDGS**YSTFGE** RYGR**NQGN**TW **LTA**FVL**KTF**A
 1051 QARAYIFIDE AHITQAL**IWL** SQRQK**DNGCF** R**SSG**SLL**NNA** IKGGVEDEVT
 1101 LSAYIT**IALL** EIPLTVTHPV VR**NALF**C**LES** **AWK**TAQEGDH GSHVY**T**K**ALL**
 1151 **AYAFAL**AGNQ **DKR**KEVLKSL NEEAVK**KDNS** **VH**WERP**QKPK** APVGHFYEPQ
 1201 APSAEVEMTS YVLLAYLTAQ PAPT**SED**LTS ATNIVK**WITK** **QQNA**QGG**FSS**
 1251 **TQDTV**VALHA **LSKY**GAATFT RT**GKAAQ**VTI **QSSG**TFSS**KF** QVDNN**N**RL**LL**
 1301 **QQVSL**PELPG EYS**MK**VTGEG CVYLQ**T**SLKY **NIL**PEKE**EF**P **FAL**GVQ**T**LPQ
 1351 **TCDEP**KAHTS **FQIS**LSVSYT **GSR**SAN**MAI** **VD**VKM**VSG**FI **PL**KPT**V**K**M**LE
 1401 RSNHVS**RTEV** SS**NH**VLIYLD **KVS**NQ**TLSL**F **FT**VLQ**D**VP**V**R DLKPA**I**V**K**VY
 1451 **DYYET**DEF**AI** **AEYN**AP**CSK**D LGNA

Excision iii)

Match to: **A2MG_HUMAN** Score: **179** Expect: **2.5e-14**
Alpha-2-macroglobulin OS=Homo sapiens GN=A2M PE=1 SV=3
Nominal mass (M_r): **164613**; Calculated pI value: **6.03**
NCBI BLAST search of [A2MG_HUMAN](#) against nr
Unformatted [sequence string](#) for pasting into other applications
Taxonomy: [Homo sapiens](#)
Fixed modifications: Carbamidomethyl (C)
Variable modifications: Oxidation (M)
Cleavage by Trypsin: cuts C-term side of KR unless next residue is P
Number of mass values searched: **53**
Number of mass values matched: **25**
Sequence Coverage: **29%**

Matched peptides shown in **Bold Red**

```

1 MGKNKLLHPS  LVLLLLVLLP  TDASVSGKPQ  YMVLVPSLLH  TETTEKGCVL
51 LSYLNETVTV  SASLESVRGN  RSLFTDLEAE NDVLHCVAFA VPKSSSNEEV
101 MFLTQVQKGP  TQEFKRTTV  MVKNEDSLVF  VQTDKSIYKP  GQTVKFRVVS
151 MDENFHPLNE LIPLVYIQDP KGNRIAQWQS FQLEGGLKQF SFPLSSEFPQ
201 GSYKVVVQKK SGGRTEHPFT VEEFVLPKFE  VQTVPKIIT  ILEEEMNVSV
251 CGLTYGKPV  PGHVTVSICR  KYSDASDCHG  EDSQAFCEKF  SGQLNSHGCF
301 YQQVKTKVFQ  LKRKEYEMKL  HTEAQIQEEG  TVVELTGRQS  SEITRITIKL
351 SFVKVDShFR  QGIPFFGQVR  LVDGKGVPIP  NKVIFIRGNE  ANYYSNATTD
401 EHGLVQFSIN  TTNVMGTSLT  VRVNYKDRSP  CYGYQWVSEE  HEEAHHTAYL
451 VFSPSKSFVH  LEPMSHELPC  GHTQTVQAHY  ILNGGTLGL  KKLSFYYLIM
501 AKGGIVRTGT  HGLLVKQEDM  KGHFSISIPV  KSDIAPVARL  LIYAVLPTGD
551 VIGDSAKYDV  ENCLANKVDL  SFSPSQSLPA  SHAHLRVTA  PQSVCALRAV
601 DQSVLLMKPD  AELSASSVYN  LLPEKDLTGF  PGPLNDQDNE  DCINRRNVYI
651 NGITYTPVSS  TNEKDMYSFL  EDMGLKAFTN  SKIRKPKMCP  QLQQYEMHGP
701 EGLRVGFYES  DVMGRGHARL  VHVEEPHTET  VRKYFPETWI  WDLVVVNSAG
751 VAEVGVTVPD  TITWKAAGAF  CLSEDAGLGI  SSTASLRAFQ  PFFVELTMPY
801 SVIRGEAFTL  KATVLNLYPK  CIRVSVQLEA  SPAFLAVPVE  KEQAPHCICA
851 NGRQTVSWAV  TPKSLGNVNF  TVSAEALESQ  ELCGTEVPSV  PEHGRKDTVI
901 KPLLVEPEGL  EKETTFNSLL  CPSGGEVSEE  LSLKLPPNVV  EESARASVSV
951 LGDILGSAMQ  NTQNLQMPY  GCGEQNMVLF  APNIYVLDYL  NETQQLTPEI
1001 KSKAIGYLN  GYQRQLNYKH  YDGSYSTFGE  RYGRNQNTW  LTAFVLKTFA
1051 QARAYIFIDE  AHITQALIWL  SQRQKNGCF  RSSGSLNNA  IKGGVEDEV
1101 LSAYITIAL  EIPLTVTHPV  VRNALFCLES  AWKTAQEGDH  GSHVYTKALL
1151 AYAFALAGNQ  DKRKEVLKSL  NEEAVKKNDS  VHWERPQKPK  APVGHFYEPQ
1201 APSAEVEMTS  YVLLAYLTAQ  PAPTSEDLTS  ATNIVKWITK  QQNAQGGFSS
1251 TQDTVVALHA  LSKYGAATFT  RTGKAAQVTI  QSSGTFSSKF  QVDNNNRLLL
1301 QQVSLPELPG  EYSMKVTGEG  CVYLQTSKY  NILPEKEEFP  FALGVQTLPO
1351 TCDEPKAHTS  FQISLSVSYT  GSRSASNMAI  VDKMVSQGI  PLKPTVKMLE
1401 RSNHVSRTTEV  SSNHVLIYLD  KVSNQTLSLF  FTVLQDVPVR  DLKPAIVKVY
1451 DYETDEFAI  AEYNAPCSKD  LGNA

```

Excision iv)

Match to: **CO3_HUMAN** Score: **141** Expect: **1.6e-10**
Complement C3 OS=Homo sapiens GN=C3 PE=1 SV=2
Nominal mass (M_r): **188569**; Calculated pI value: **6.02**
NCBI BLAST search of [CO3_HUMAN](#) against nr
Unformatted [sequence string](#) for pasting into other applications
Taxonomy: [Homo sapiens](#)
Fixed modifications: Carbamidomethyl (C)
Variable modifications: Oxidation (M)
Cleavage by Trypsin: cuts C-term side of KR unless next residue is P
Number of mass values searched: **100**
Number of mass values matched: **37**
Sequence Coverage: **30%**

Matched peptides shown in **Bold Red**

1 MGPTSGPSLL LLLLTHLPLA LGSPMYSIIT PNILRLESEE TMVLEAHDAQ
51 GDVPVTVTVH DFPGKLVLS SEKTVLTPAT NHMGNVTFPI PANREFKSEK
101 GRNKFTVTVQA TFGTQVVEKV **VVLSLQSGYL FIQTDKTIYT** PGSTVLYRIF
151 TVNHHKLLPVG RTVMVNIENP EGIPVKQDSL SSQNQLGVLP LSWDIPELVN
201 MGQWKIR**AYY ENSPQQVFST EFEVKEYVLP SFEVIVEPTE** KFYIYNEKG
251 LEVTITARFL YGK**KVEGTAF VIFGIQDGEQ** RISLPESLKR IPIEDGSSEV
301 VLSRQVLLDG VQNPRADLV GKSLYVSATV ILHSGSDMVQ AER**SGIPIVT**
351 **SPYQIHFTKT PKYFKPGMPF DLMVFVTNPD GSPAYRVPVA** VQGEDTVQSL
401 TQGDGVAKLS **INTHPSQKPL SITVTRTKKQE** LSEAEQATRT **MQUALPYSTVG**
451 **NSNNYLHLSV LRTELRPGET LNVNFLLRMD RAHEAKIRYY** TYLIMNKGRLL
501 LKAGRQVREP **GQDLVVLPLS ITTDFIPSPFR LVAYYTLIGA** SQREVVADS
551 VLDRDVHDSVC GSLVVKSGQS EDRQPVPGQQ MTLKIEGDHG ARVVLLVAVDK
601 GVFVLNKKNK LTQSKIWDVV EK**ADIGCTPG SGKDYAGVFS** DAGLTFSTSS
651 **GQQTARQRAEL QCPQPAARRR** RSVQLTEKRM DKVKGYPKEL RKCCEDGMRE
701 NPMRFSCQRR TRFISLGEAC **KKVFLDCCNY ITELRRQHAR** ASHLGLARSN
751 **LDEDIAEEN IVSRSEFPES WLWNVEDLKE PPKNGISTKL** MNIFLKDSIT
801 **TWEILAVSMS DKKGICVADP** FEVTVMQDFE IDLRLPYSVV RNEQVEIRAV
851 LYNYRQNEQL **KVRVELLHNP AFCSLATTKR** RHQQTVTIPP KSLSLSPYVI
901 VPLKTGLQEV EVKAAVYHHF ISDGVKSLK VVEGIRMNK TVAVRTLDP
951 RLGR**EGVQKE DIPPADLSDQ VPDTESETRI LLQGTTPVAQM** TEDAVDAERL
1001 **KHLIVTPSGC GEQNMIGMTP** TVIAVHYLDE TEQWEKFGLE KRQGALELIK
1051 KGYTQQLAFR QPSSAFAAFV KRAPSTWLTA YVVKVFLAV NLIAIDSQVL
1101 CGAVKWLILE **KQKPDGVFQE DAPVIHQEMI GGLRNNNEKD** MALTAFLVIS
1151 LQEAKDICEE QVNSLPGSIT **KAGDFLEANY MNLQRSTYVA** IAGYALAQMG
1201 **RLKGPLLNKF LTTAKDKNRW** EDPGKQLYV EATSALLAL LQLKDFDFVP
1251 PVVRWLNEQR YGGGYGSTQ ATFMVFQALA QYQ**KDAPDHQ ELNLDVSLQL**
1301 **PSRSSKITHR IHWESASLLR** SEETKENEGF TVTAEGKQG TLSVVTMYHA
1351 KAKDQLTCNK FDLKVTIKPA PETEKRPQDA KNTMILEICT RYRGDQDATM
1401 SILDISMMTG FAPDTHDLKQ LANGVDYRIS KYELDKAFSD RNTLIIYLDK
1451 VSHSEDDCLA **FKVHQYFNVE LIQPGAVKVY AYYNLEESCT** RFYHPEKEDG
1501 KLNKLCRDEL **CRCAEENCFT QKSDDKVTLE** ERLDKACEPG VDYVYKTRLV
1551 **KVQLSNDFDE YIMAIQTIK** SGSEDEVQVQ QRTFISPIKC REALKLEEK
1601 HYLMWGLSSD FWGKPNLSY IIGKDTWVEH WPEEDECQDE ENQKQCQDLG
1651 AFTESMVVFG CPN

Match to: **CO4A_HUMAN** Score: 60 Expect: 0.021

Complement C4-A OS=Homo sapiens GN=C4A PE=1 SV=1

Nominal mass (M_r): 194247; Calculated pI value: 6.65

NCBI BLAST search of [CO4A_HUMAN](#) against nr

Unformatted [sequence string](#) for pasting into other applications

Taxonomy: [Homo sapiens](#)

Fixed modifications: Carbamidomethyl (C)

Variable modifications: Oxidation (M)

Cleavage by Trypsin: cuts C-term side of KR unless next residue is P

Number of mass values searched: 100

Number of mass values matched: 22

Sequence Coverage: 20%

Matched peptides shown in **Bold Red**

1 MRLWGLIWA SSFFTLSQLK PRLLLFSPSV VHLGVPLSVG VQLQDVPRGQ
51 VVKGSVFLRN PSRNNVPCSP KVDFTLSSER **DFALLSLQVP LKDAKSCGLH**
101 QLLRGPEVQL VAHSPWLKDS LSR**TNIQGI NLLFSSRRGH LFLQTDQPIY**
151 **NPGQRVRYRV FALDQKMRPS** TDTITVMVEN SHGLRVRKKE VYMPSSIFQD
201 DFVIPDISEP GTWKISARFS DGLSNSSTQ FEVKKYVLPN FEVKITPGKP
251 YILTVPGHLD EMQLDIQARY **IYGKPVQGVA YVRFGLLDED** GKKTFFRGL
301 SQTKLVNGQS HISLSKAEFQ DALEKLNMGITDLQGLRLYV AAALIESPGG
351 EMEEAELTSW YFVSSPFLSD LSKTKRHLVP **GAPFLQALV** REMSGSPASG
401 IPVKVSATVS SPGSVPEVQD IQQNTDGSQ VSIPIIIPQT ISELQLSVSA
451 GSPHPAIARL **TVAAPPSGGP GFLSIERPDS RPPRVGDTLN** LNLRAVGSGA
501 **TFSHYYMIL SRGQIVFMNR** EPKRTLTSVS VFDHHLAPS FYFVAFYHGH
551 DHPVANSLRV DVQAGACEGK LELSVDGAKQ YRNGESVKLH LETDSLALVA
601 LGALDTALYA AGSKSHKPLN MGKVFEAMNS YDLGCGPGG DSALQVFQAA
651 GLAFSDGDQW TLSRRLSCLP KEKTTRKKRN VNFQKAINLK LGQYASPTAK

701 RCCQDGVTRL PMMRSCEQRA AR**VQQPDCRE** **PFLSCCQFAE** **SLRKKSRDKG**
 751 QAGLQ**RALEI** **LQEDLIDED** **DIPVRSFFPE** NWLWRVETVD RFQILTLWLP
 801 DSLTTWEIHG LSLSKTKGLC VATPVQLRVF REFHLHLRLP MSVRRFEQLE
 851 LRPVLYNYLD KNLTVSVHVS PVEGLCLAGG GGLAQQLVLP AGSARPVAFS
 901 VVPTAAAVS LKVVARGSF EFPVGDVAVSKV LQIE**KEGAIH** **REELVYELNP**
 951 **LDHRGR****TLEI** **PGNSDPNMIP** **DGDFNSYVRV** **TASDPLDTLG** **SEGALSPGGV**
 1001 **ASLLR**LPRGC GEQTMIIYLAP TLAASRYLDK TEQWSTLPPE TKDHAVDLIQ
 1051 KGYMR**LQQFR** KADGSYAOWL SRDSSTWLTA FVLKVLSLAQ EQVGGSPPEKL
 1101 QETS**NWLLSQ** QQADGSFQDP CPVLDRSMQG GLVGNDETVA LTAFTVIALH
 1151 HGLAVFQDEG AEPLKQRVEA SISKANSFLG EKASAGLLGA HAAAITAYAL
 1201 SLTKAPVDLL GVAHNNLMAM AQETGDNLYW GSVTGSQNSA VSPTPAPRNP
 1251 SDPMPQAPAL WIETTAYALL HLLLHEG**KA** **MADQASAWLT** **RQGSFQGGFR**
 1301 **STQDTVIALD** **ALSAYWIASH** **TTEERGLNVT** LSSTGRNGFK SHALQLNNRQ
 1351 IR**GLEELQF** **SLGSKINVKV** GGNSKGTLKV LRTYNVLDK NTTCQDLQIE
 1401 VTVKGHVEYT MEANEDYEDY EYDELPAKDD PDAPLQPVTP LQLFEGRNR
 1451 RRREAPKVV EQUESRVHYTV CIWRNGKVGL SGMIAADVTL LSGFHALLRAD
 1501 LEKLTSLSDR **YVSHFETEGP** **HVLLYFDSVP** **TSRECVGFEA** VQEVVGLVQ
 1551 PASATLYDYY NPERRCVYF GAPSKSR**LLA** **TLCSAEVCQC** **AEGKCPQR**
 1601 ALERGLQDED GYRMKFACY PRVEYGFQVK VLREDSRAAF RLFETKITQV
 1651 LHFTKDVKAA ANQMRNFLVR ASCRLRLEPG KEYLIMGLDG ATYDLEGHPO
 1701 YLLDSNSWIE EMPSERLCRS TRQR**ACAQL** **NDFLQEYGTQ** **GCQV**

Match to: **CO4B_HUMAN** Score: **68** Expect: **0.0036**

Complement C4-B OS=Homo sapiens GN=C4B PE=1 SV=1

Nominal mass (M_r): **194212**; Calculated pI value: **6.73**

NCBI BLAST search of [CO4B_HUMAN](#) against nr

Unformatted [sequence string](#) for pasting into other applications

Taxonomy: [Homo sapiens](#)

Fixed modifications: Carbamidomethyl (C)

Variable modifications: Oxidation (M)

Cleavage by Trypsin: cuts C-term side of KR unless next residue is P

Number of mass values searched: **100**

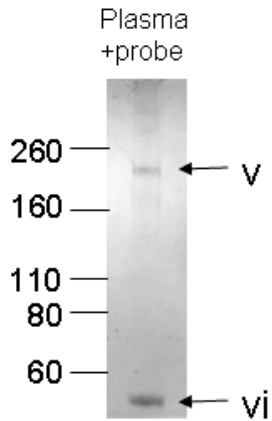
Number of mass values matched: **23**

Sequence Coverage: **21%**

Matched peptides shown in **Bold Red**

1 MRLLLWGLIWA SSFFTL**SLQK** PRLLLFSPSV VHLGVPLSVG VQLQDVPRGQ
 51 VVKGSVFLRN PSRNNVPCSP KVDFTLSSER **DFALLSLQVP** **LKDAK**SCGLH
 101 QLLRGPEVQL VAHSPWLKDS LSRT**TNIQGI** **NLLFSSRRGH** **LFLQTDQPIY**
 151 **NPGQVRVYRV** FALDQKMRPS TDTITVMVEN SHGLRVRKKE VYMPSSIFQD
 201 DFVIPDISEP GTWKISARFS DGLESNSSTQ FEVKKYVLPN FEVKITPKKP
 251 YILTPVPHLD EMQLDIQAR**Y** **IYGKPVQGVA** **YVRFGLLDED** GKKTFFRGLE
 301 SQTKLNGQS HISLSKAEFQ DALEKLNMGITDLQGLRLYV AAAIIESPGG
 351 EMEEAELTSW YFVSSPFFSLD LSKTKRHLVP **GAPFLQALV** REMSGSPASG
 401 IPVKVSATVS SPGSVPEVQD IQQNTDGGSGQ VSIPIIIIPQT ISELQLSVSA
 451 GSPHPAIARL **TVAAPPSGGP** **GFLSIERPDS** **RPPRVGDTLN** LNLRAVGSGA
 501 **TFSHYYMIL** SRGQIVFMNR EPKRTLTSVS VFVDHHLAPS FYFVAFYYHG
 551 DHPVANSLRV DVQAGACEGK LELSVDGAKQ YRNGESVKLH LETDSLALVA
 601 LGALDTALYA AGSKSHKPLN MGKVFEAMNS YDLGCGPGGG DSALQVFQAA
 651 GLAFSDGDQW TLSRKRRLSCP KEKTRKKRN VNFQKAIN EK LGQYASPTAK
 701 RCCQDGVTRL PMMRSCEQRA AR**VQQPDCRE** **PFLSCCQFAE** **SLRKKSRDKG**
 751 QAGLQ**RALEI** **LQEDLIDED** **DIPVRSFFPE** NWLWRVETVD RFQILTLWLP
 801 DSLTTWEIHG LSLSKTKGLC VATPVQLRVF REFHLHLRLP MSVRRFEQLE
 851 LRPVLYNYLD KNLTVSVHVS PVEGLCLAGG GGLAQQLVLP AGSARPVAFS
 901 VVPTAAAVS LKVVARGSF EFPVGDVAVSKV LQIE**KEGAIH** **REELVYELNP**
 951 **LDHRGR****TLEI** **PGNSDPNMIP** **DGDFNSYVRV** **TASDPLDTLG** **SEGALSPGGV**
 1001 **ASLLR**LPRGC GEQTMIIYLAP TLAASRYLDK TEQWSTLPPE TKDHAVDLIQ
 1051 KGYMR**LQQFR** KADGSYAOWL SRDSSTWLTA FVLKVLSLAQ EQVGGSPPEKL
 1101 QETS**NWLLSQ** QQADGSFQDL **SPVIHRSMQG** GLVGNDETVA LTAFTVIALH
 1151 HGLAVFQDEG AEPLKQRVEA SISKANSFLG EKASAGLLGA HAAAITAYAL
 1201 SLTKAPVDLL GVAHNNLMAM AQETGDNLYW GSVTGSQNSA VSPTPAPRNP
 1251 SDPMPQAPAL WIETTAYALL HLLLHEG**KA** **MADQASAWLT** **RQGSFQGGFR**
 1301 **STQDTVIALD** **ALSAYWIASH** **TTEERGLNVT** LSSTGRNGFK SHALQLNNRQ
 1351 IR**GLEELQF** **SLGSKINVKV** GGNSKGTLKV LRTYNVLDK NTTCQDLQIE
 1401 VTVKGHVEYT MEANEDYEDY EYDELPAKDD PDAPLQPVTP LQLFEGRNR
 1451 RRREAPKVV EQUESRVHYTV CIWRNGKVGL SGMIAADVTL LSGFHALLRAD
 1501 LEKLTSLSDR **YVSHFETEGP** **HVLLYFDSVP** **TSRECVGFEA** VQEVVGLVQ

1551 PASATLYDYY NPERRCVVFY GAPSKSRLLA **TLCSAEVCQC** **AEGK**CPRQRR
 1601 ALERGLQDED GYRMKFACY Y PRVEYGFQVK VLREDSRAAF RLFETKITQV
 1651 LHFTKDVKAA ANQMRNFLVR ASCRLRLEPG KEYLIMGLDG ATYDLEGHPO
 1701 YLLDSNSWIE EMPSERLCRS TRQRAACAQL **NDFLQEYGTQ** **GCQV**



Excision vi)

Match to: **ALBU_HUMAN** Score: 153 Expect: 1e-11
Serum albumin OS=Homo sapiens GN=ALB PE=1 SV=2
 Nominal mass (M_r): 71317; Calculated pI value: 5.92
 NCBI BLAST search of [ALBU_HUMAN](#) against nr
 Unformatted [sequence string](#) for pasting into other applications
 Taxonomy: [Homo sapiens](#)
 Fixed modifications: Carbamidomethyl (C)
 Variable modifications: Oxidation (M)
 Cleavage by Trypsin: cuts C-term side of KR unless next residue is P
 Number of mass values searched: 79
 Number of mass values matched: 21
 Sequence Coverage: 47%

Matched peptides shown in **Bold Red**

1 MKWVTFISLL FLFSSAYSRG VFRDAHKSE VAHRFKDLGE ENFK**ALV**LIA
 51 **FAQYLQ**CPF **EDHV**KLNVN TEFAKTCVAD ESAENC**DKSL** **HTLFGDKLCT**
 101 **VATLR**ETIGE MADCCAQEP ERNECFLOHK DDNP**NLPRLV** **RPEVDVMCTA**
 151 **FHDNEETFLK** **KYL**EIARRH **PYFYAPELLF** **FAKRYKAAFT** **ECCQAADKAA**
 201 **CLLPK**LDELRL DEGKASSAQ RLK**CASLQKF** GERAFKAWAV ARLSQRFPKA
 251 EFAEVSKLVT DLTK**VHTECC** **HGDLL**ECADD **RADLAKYICE** NQDSISSKLLK
 301 ECCEKPLLEK **SHCIAEVEND** **EMPADLPSLA** **ADFVESKDVC** **KNYAEAKDVF**
 351 **LGMFLY**EAR RHPDYSV**VLL** **LRLAKTYETT** LEKCCAAADP HECYAK**VFDE**
 401 **FKPLV**E**EPQN** **LIKQNC**ELFE **QLGEYKFQNA** **LLVRYTKKVP** **QVSTPTLVEV**
 451 **SRNLG**KVGSK CCKHPEAK**RM** **PCAEDYLSVV** **LNQLCVLHEK** TPVSDRVTKC
 501 CTESLVNRRP **CFSAL**EVDET **YVPKEFNAET** **FTFHADICTL** **SEKERQIKKQ**
 551 TALVELVKHK PKATKEQLKA VMDDFAAFVE KCKKADDKET CFAE**E**GKLLV
 601 AASQAALGL