

Fig. 1

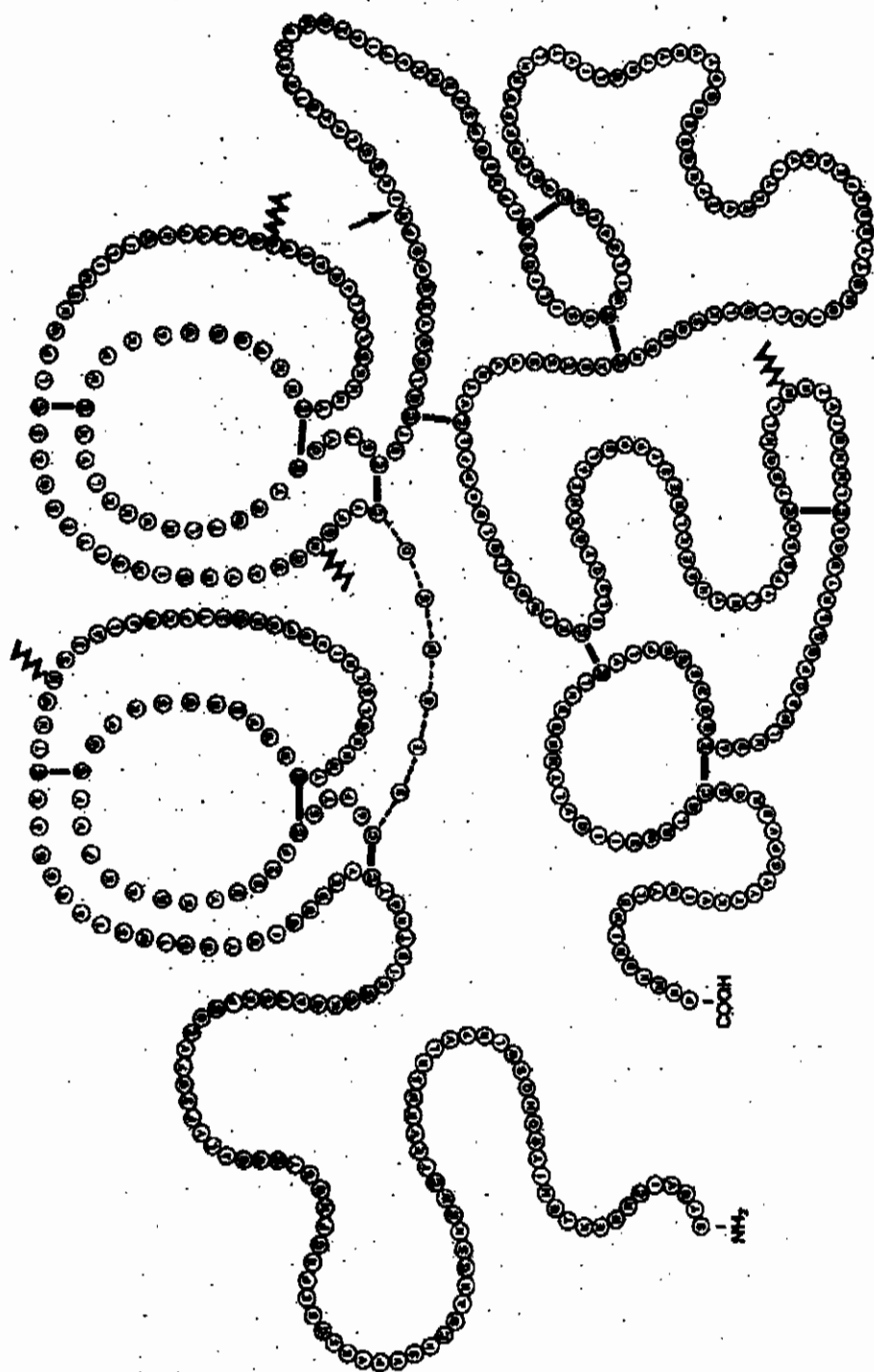


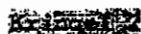
Fig. 2b

[GENETYX-MAC: Multiple Alignment]

Date : 2003.08.23

tPA Isoform 1 vs DSPA alpha 1

tPA_AS	1	M-DAMKRGGLCCVLLLCGAVFVSPSQEIHFRRGARSYQVICRDEKTMIIYQQHQSWLRP	59
DSPA_AS	1	MVNTMTKTKLLCVLLLCGAVFSLFRQETRYQLARGSRAYGVACKDEITQMTYRRQESWLRP	60
		* * * * *	
tPA_AS	60	VLRNVRVEYCWNSGRAQCHSVPVKSCSEPRCFNGGTCQQALYFSDFCQCEGFAAGKCC	119
DSPA_AS	61	EVRSKRVEHCQCDRGQARCHTVFVNSCSEPRCFNGGTCWQAVYFSDFCQCPAGYTGRKC	120
		* * * * *	
tPA_AS	120	EIDTRATCYEDQGISYRGTWSTAESGAECTNWNSSALAQKPYSGRRPDAILGLGNHNYC	179
DSPA_AS	121	EVDTRATCYEGQGVYRGTWSTAESRVECINWNSSLLTRTYNGRMPDAFNLGLGNHNYC	180
		* * * * *	
tPA_AS	180	RNPDRDSKPWCYVFKAGKYSSEFCSTPACSEGNDC	239
DSPA_AS	181	RNPNGAPKWCYVIKAGKFTSESCSVVPCSK-----	204
		* * * * *	
tPA_AS	240	-----	299
DSPA_AS	205	-----ATC	214
		* *	
tPA_AS	300	GLRQYSQPFKGGLEFADIASHPWQAIFAKHRRSPGERFLCGGILISSCWILSAAHCF	359
DSPA_AS	215	GLRKYKEPQLHSTGGGLFTDITSHPWQAIFAQNRSSGERFLCGGILISSCWVLTAAHCF	274
		* * * * *	
tPA_AS	360	QERFPPHHLTIVLGRTRYVVPGEESQKFEVEKYIVHKEFDDDTYNDIALQLKSDSSRC	419
DSPA_AS	275	QESYLPDQLKVVLGRTRYVKPGEEETFKVKKYIVHKEFDDDTYNNDIALQLKSDSPQC	334
		* * * * *	
tPA_AS	420	AQESSVVRTVCLPPADLQLPDWTECELSGYGKHEALSPFYSERLKEAHVRLYPSSRCTSQ	479
DSPA_AS	335	AQESDSVRAICLPEANLQLPDWTECELSGYGKHKSSSPFYSEQLKEGHVRLYPSSRCAPK	394
		* * * * *	
tPA_AS	480	HLLNRTVTDNMLCAGDTRSGGPQANLHDACQGDSSGGLVCLNDGRMTLVGIIISWGLGCGQ	539
DSPA_AS	395	FLFNKTVTNNMLCAGDTRSGEIYPNVHDACQGDSSGGLVCMNDNHMTLLGIISWGVGCGE	454
		* * * * *	
tPA_AS	540	KDVPGVYTKVTNYLDWIRDNMRP	562
DSPA_AS	455	KDVPGVYTKVTNYLGWIRDNMHL	477
		* * * * *	




Humanised DSPA

a) Deletion of Kringel 2

b) tPA activation site mutated to corresponding DSPA sequence

```

1 mdamkrglcc villcgavfv spsqeiharf rrgarsyqvi crdektqmiy qqhgswlrpv
61 lrsnrveycw cnsgraqchs vpvkscsepr cfnggtcqqa lyfsdfvcqc pegfagkcce
121 idtratcyed qgisyrgrws taesgaectn wnssalagkp ysgrrpdair lglgnhnycr
181 npdrdskpw cyvfkgagky ssefcstpa csegfagkq glrqysqp qf█████████ kgglf
241 adiaashpwq aaifakhrr spgerflcg gilisscwi lsaahcfqe rfpphhlvt
301 ilgrrtyrvv pgeeeqkfe vekiivhke fdddtydnd iallqlksd ssrcaqess
361 vvrvtclpp adlqlpdwt ecelsgygk healspfys erlkeahvr lypssrcts
421 qhllnrtvt dnmclcagdt rsggpqanl hdacqgdsq gplvclndg rmtlvgiis
481 wqlgcgqkd vpgvytkvt nyldwirdn mrp

```

Italics: Signal sequence

Kringel 2 Deletion: ~~nsd-Kringel2-sec~~

Conversion of OD405 into converted substrate

$$[\text{Flavigen, } \mu\text{M}] = (\text{OD} - 0.012)/0.00298$$

$$R^2 = 0.9996$$

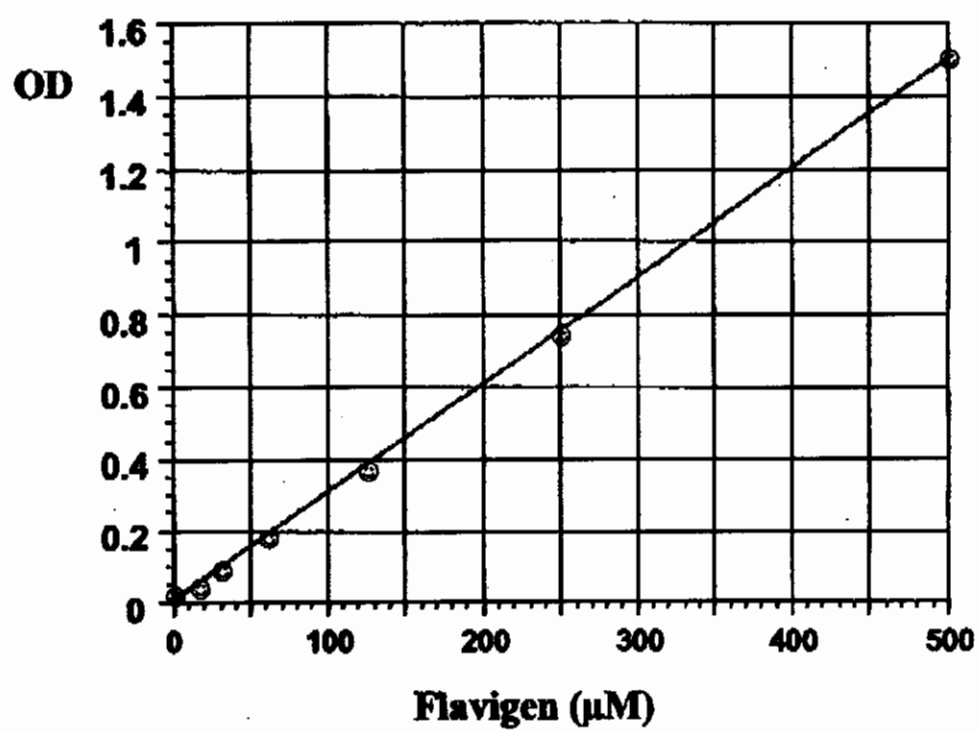


Figure 4a

Conversion of Flavigen.pli by 10 nM plasmin

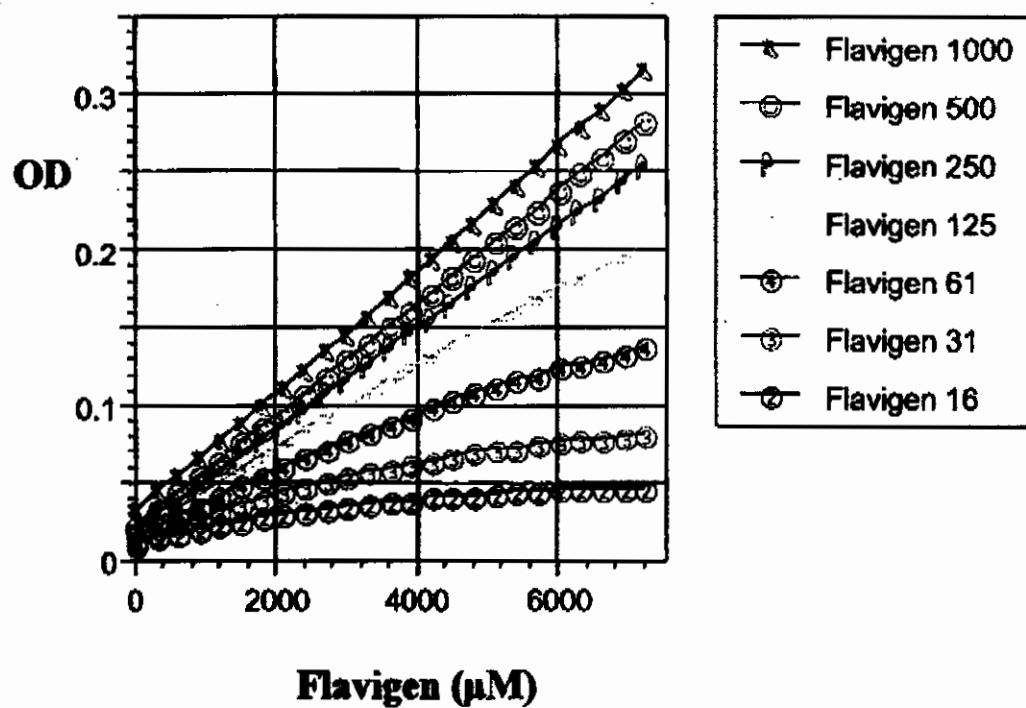


Figure 4b

Plot of pNA generation (nM/s) by 10 nM of plasmin as a function of Flavigen.pli concentration
 $k_{cat} = 1.36 \text{ s}^{-1}$; $K_m = 88.1 \text{ } \mu\text{M}$

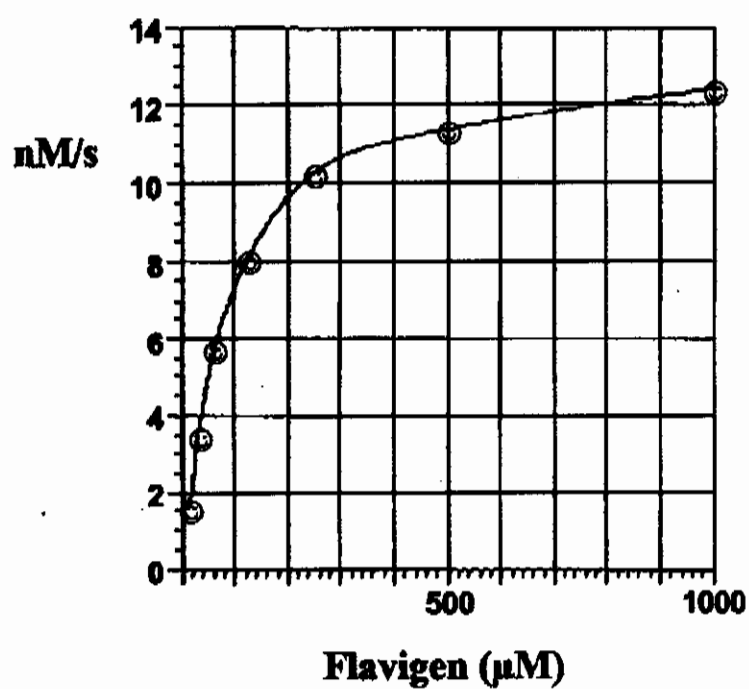
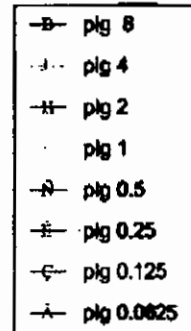
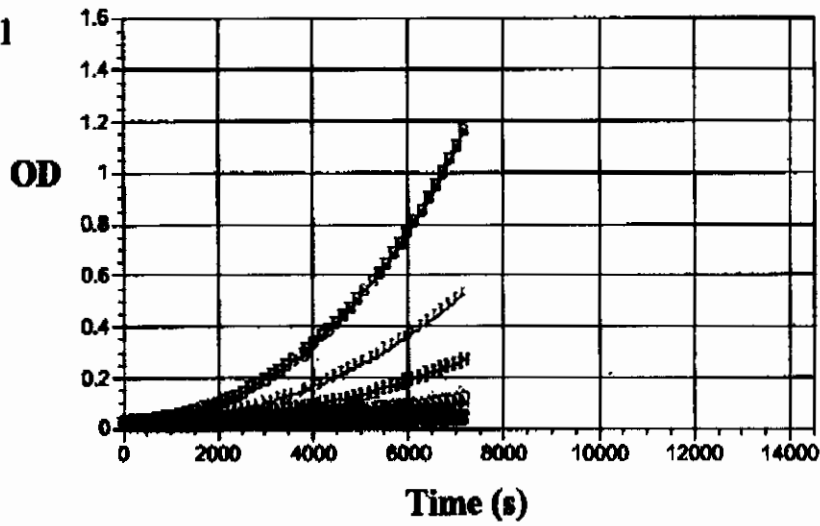


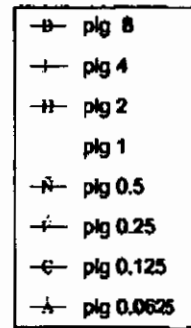
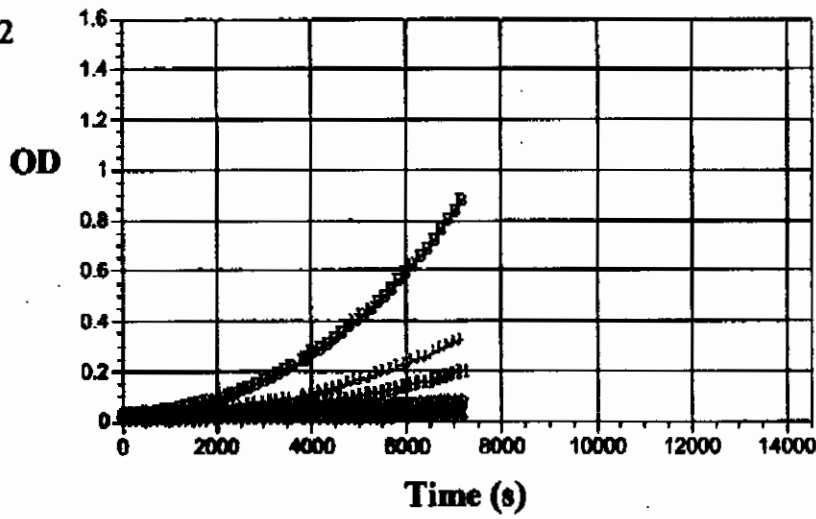
Figure 4c

t-PA alone no cofactor

exp. 1



exp. 2



exp. 3

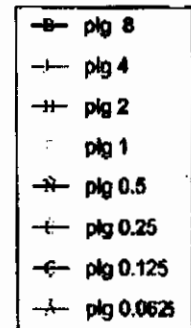
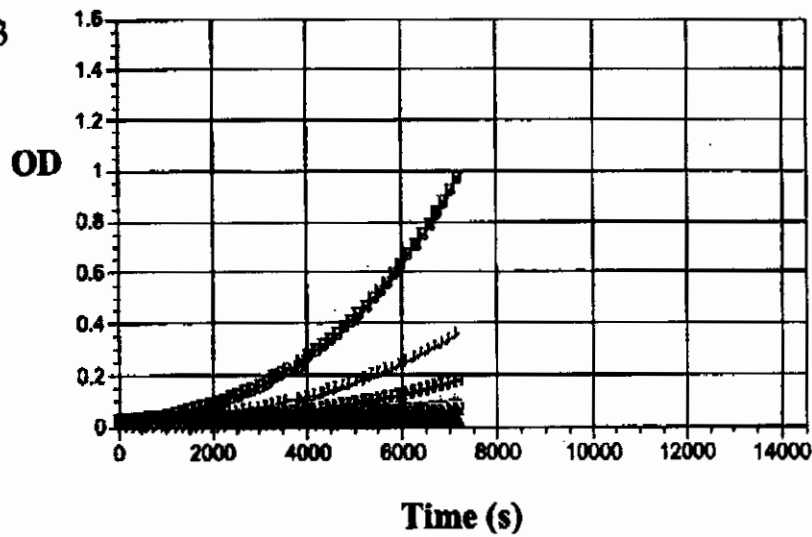
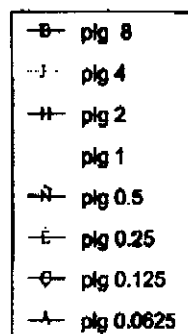
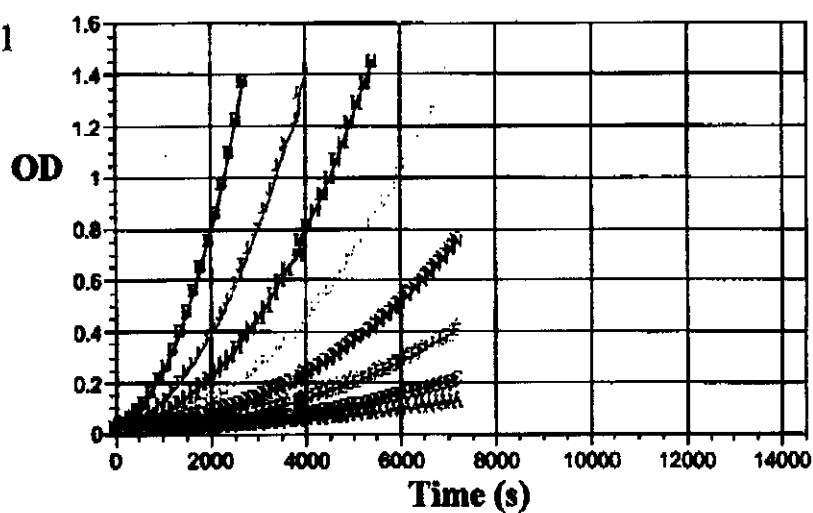


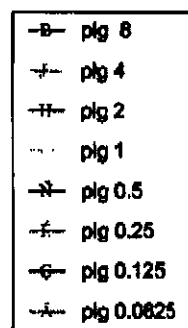
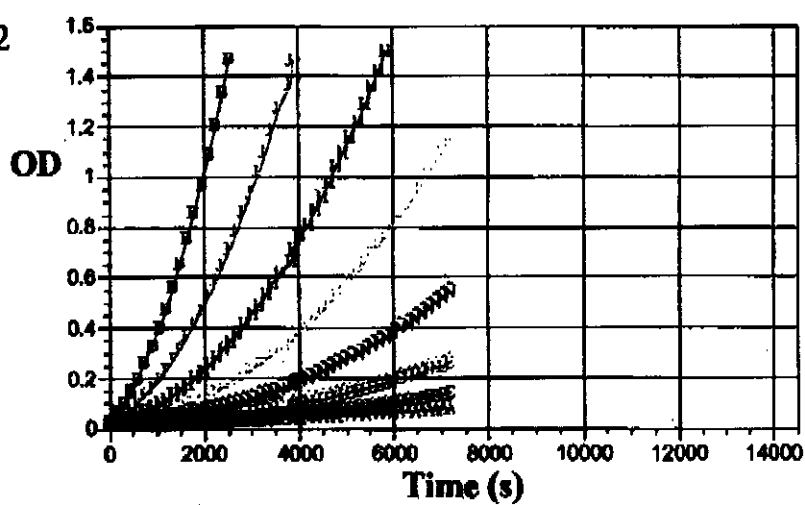
Figure 5a

t-PA , b-amyloid

exp. 1



exp. 2



exp. 3

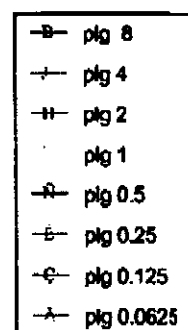
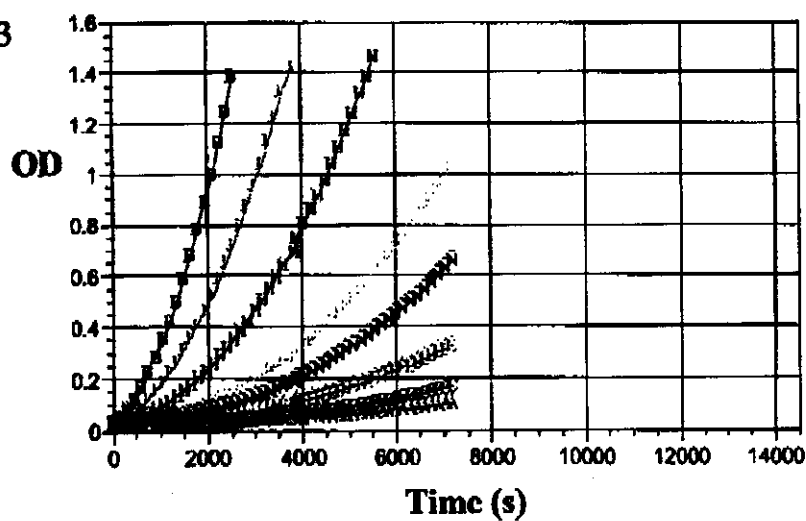
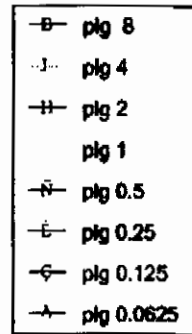
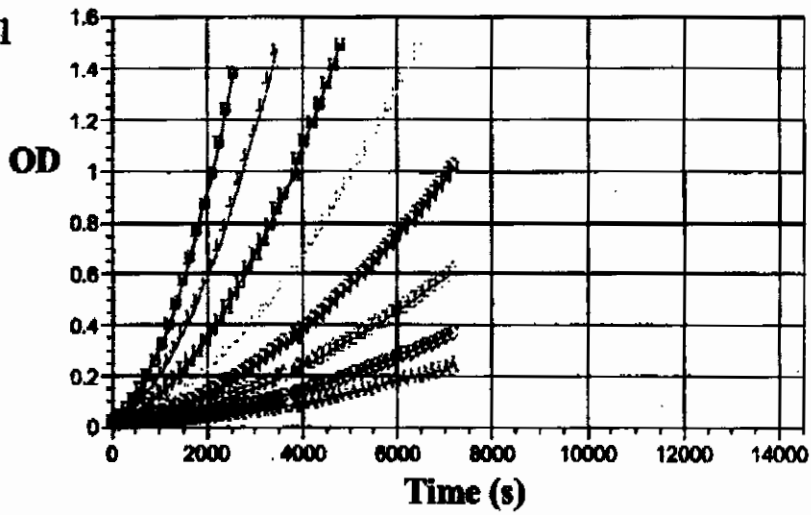


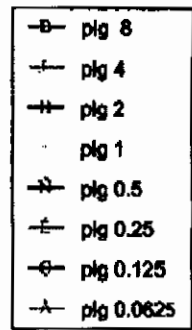
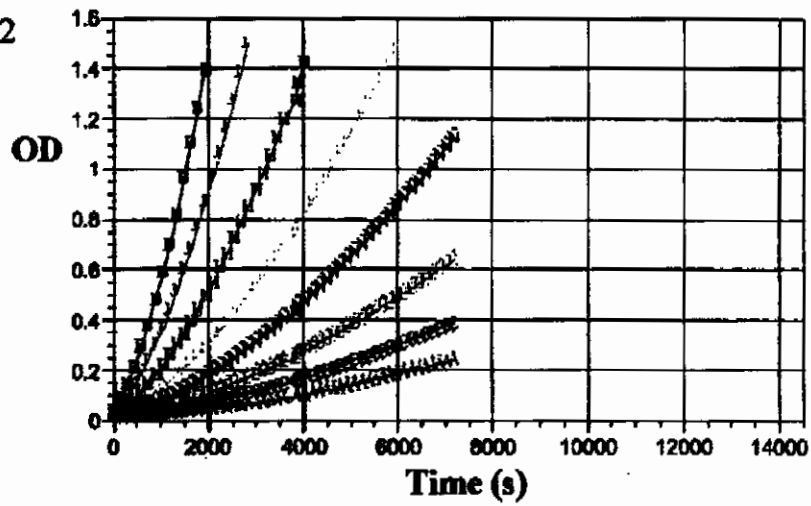
Figure 5b

t-PA , fibrinogen

exp. 1



exp. 2



exp. 3

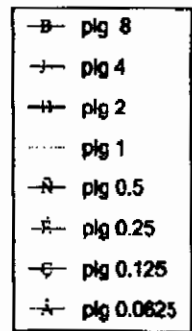
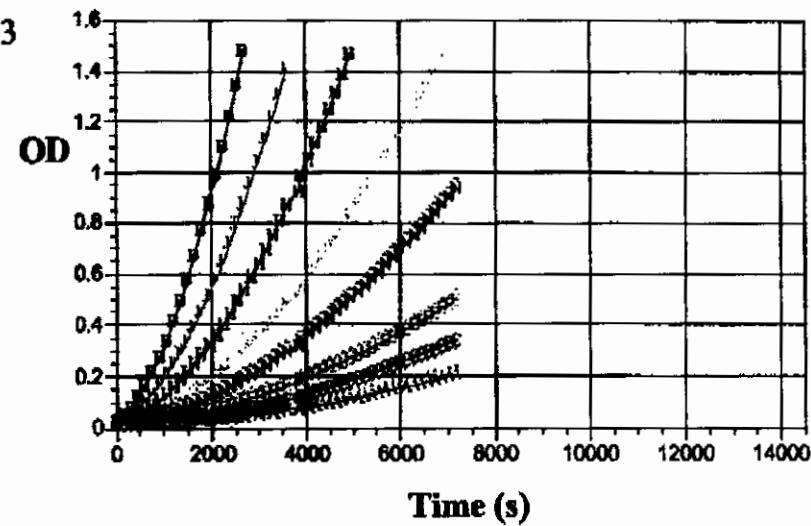
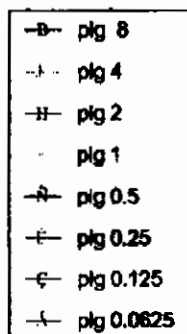
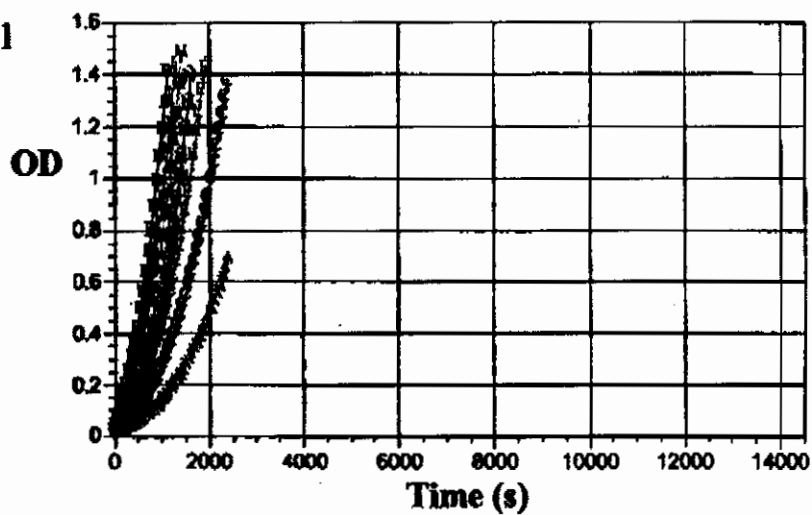


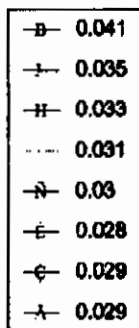
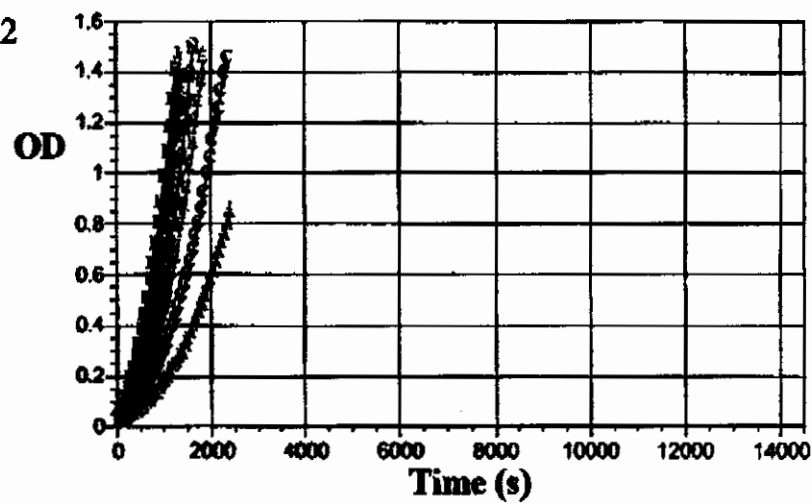
Figure 5c

t-PA, fibrin

exp. 1



exp. 2



exp. 3

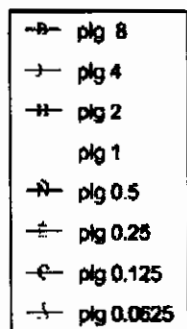
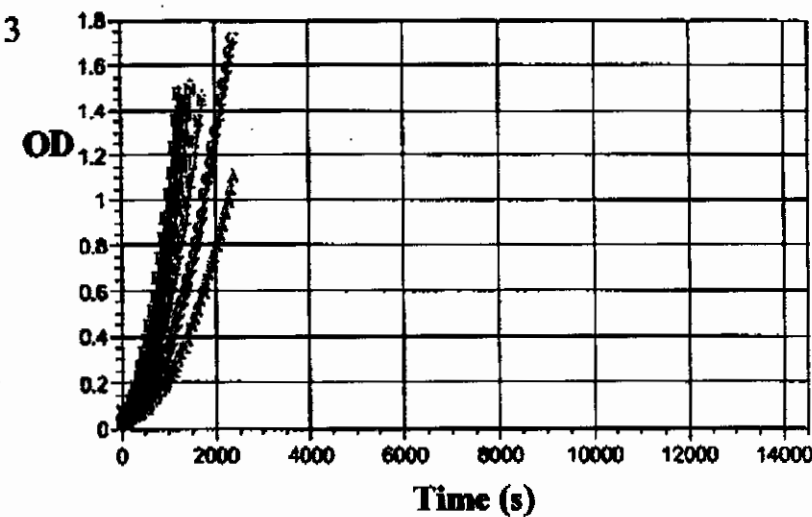
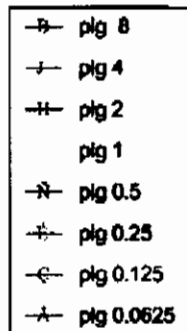
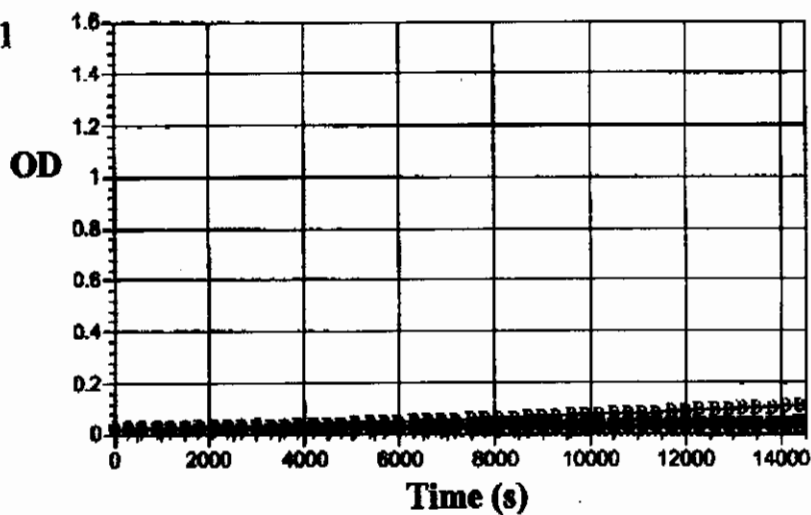


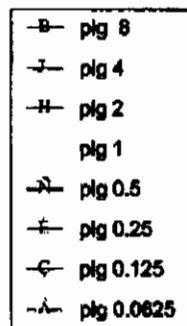
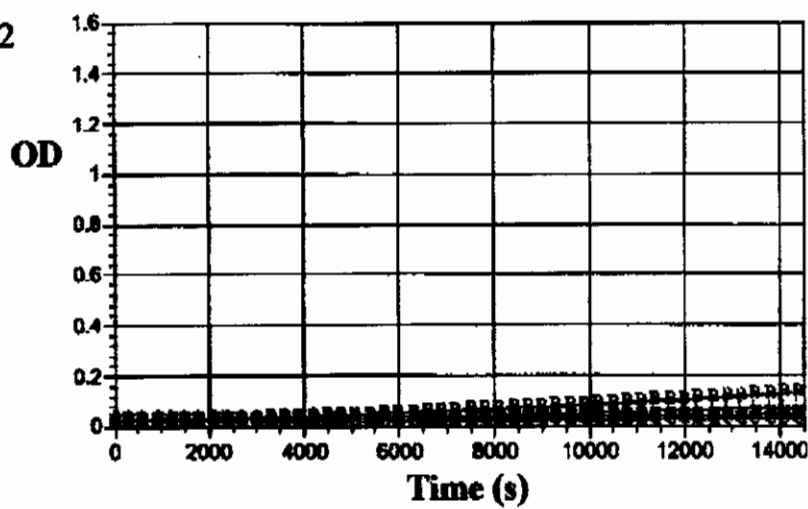
Figure 5d

DSPA, no cofactor

exp. 1



exp. 2



exp. 3

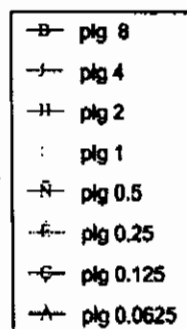
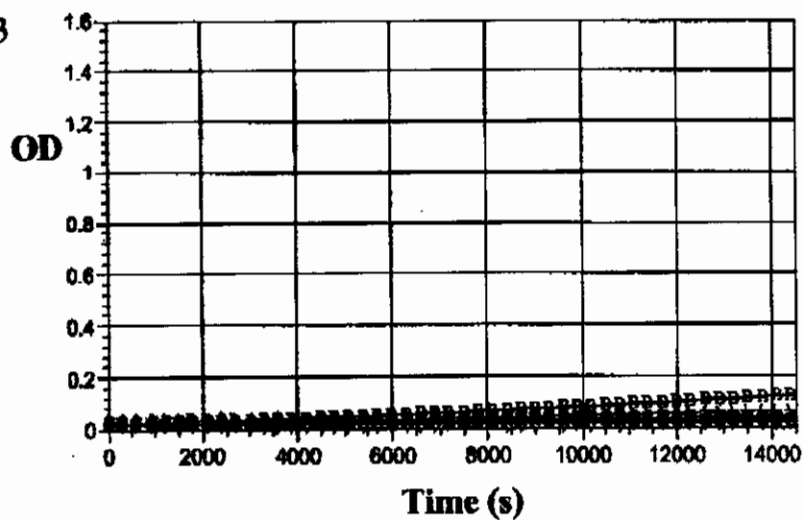


Figure 5e

DSPA, b-amyloid

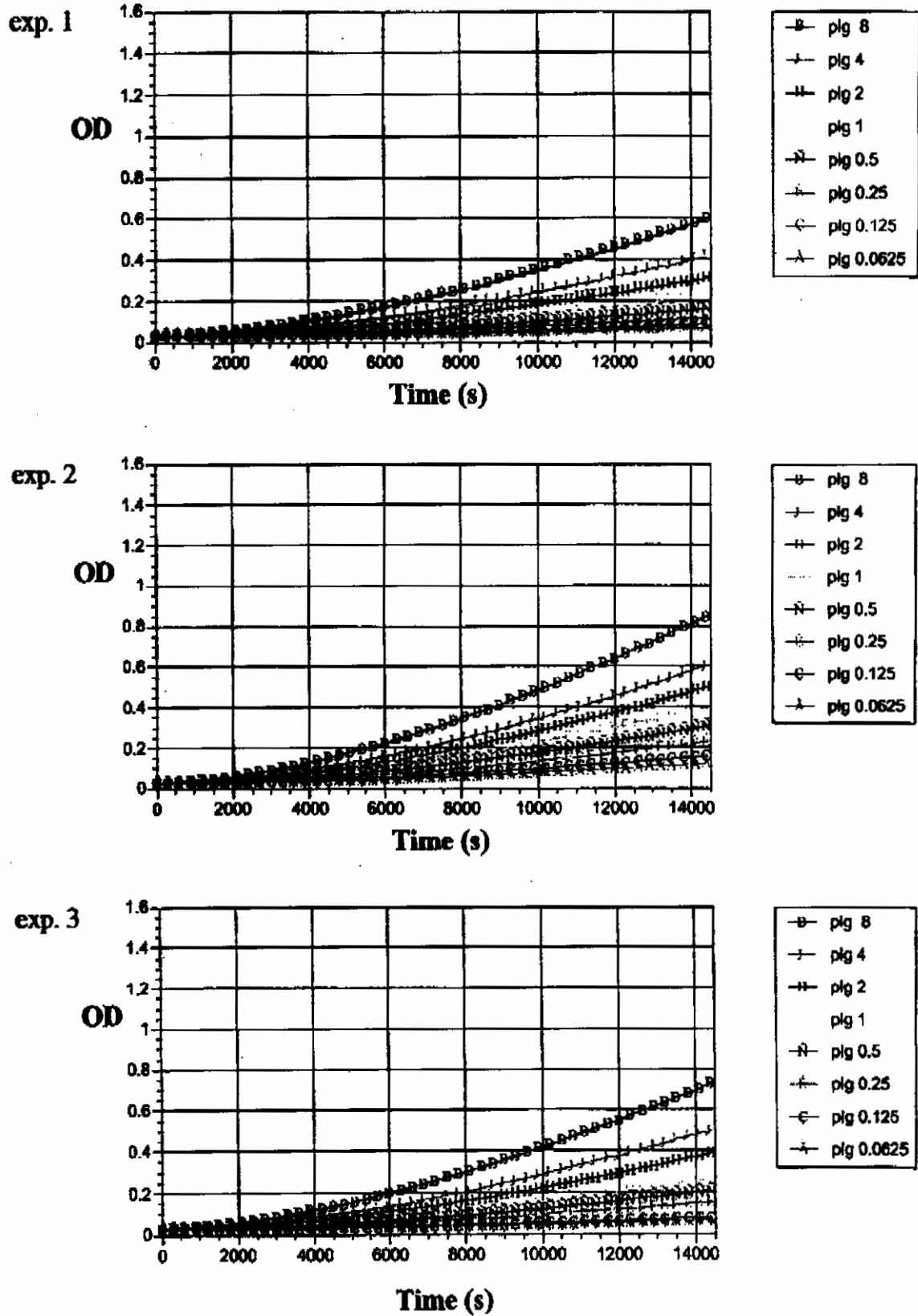
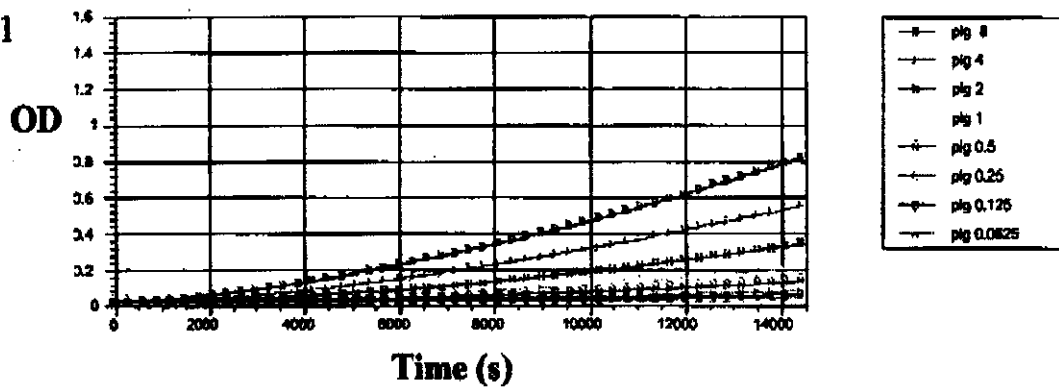


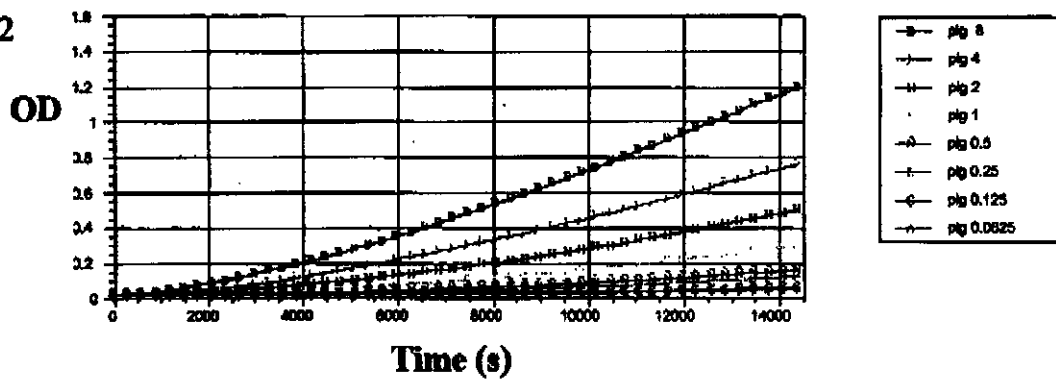
Figure 5f

DSPA, fibrinogen

exp. 1



exp. 2



exp. 3

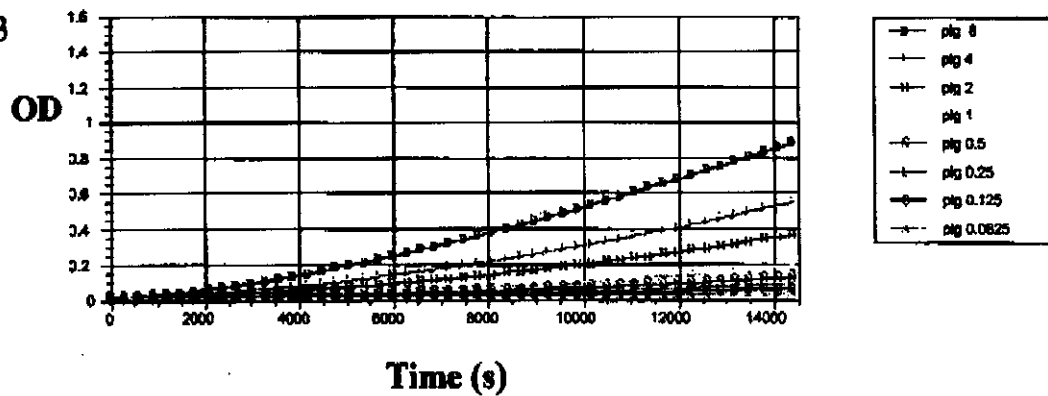
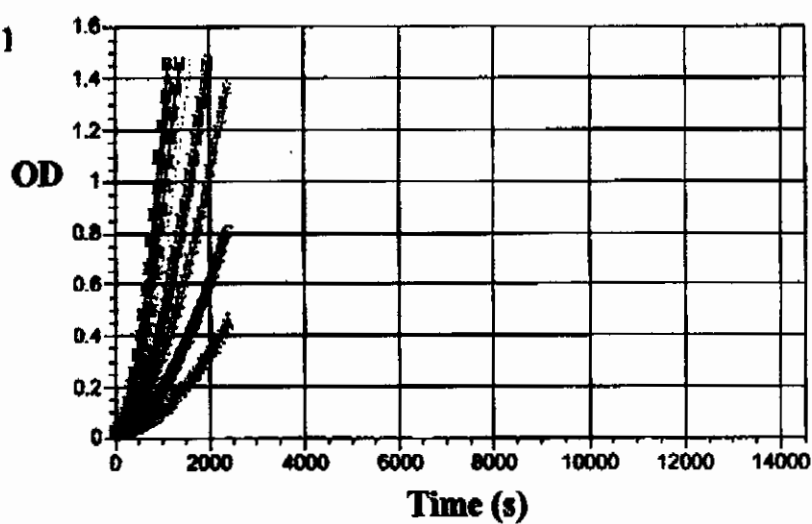


Figure 5g

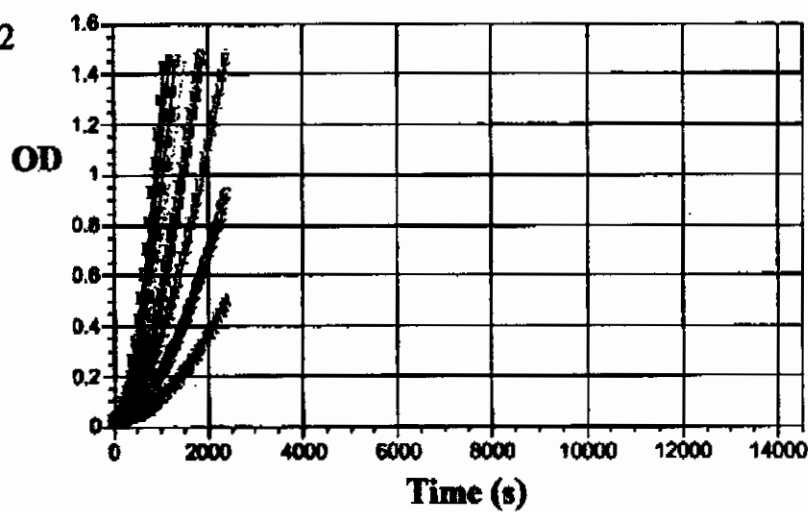
DSPA , fibrin

exp. 1



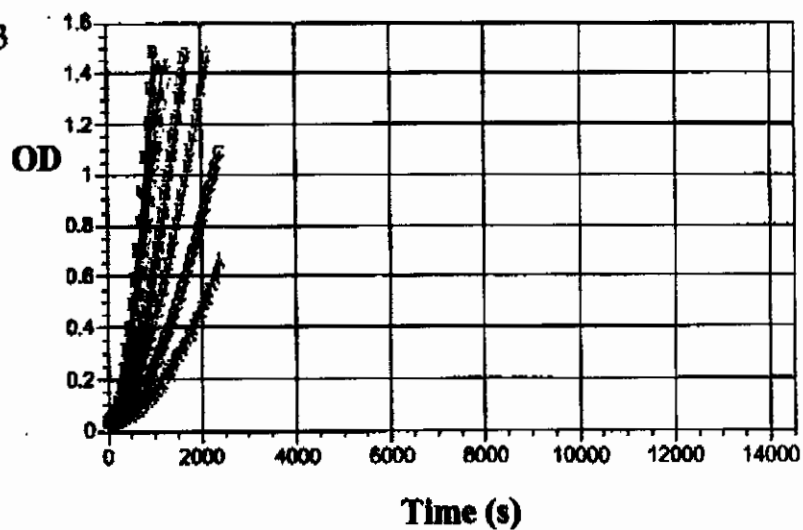
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 plg 4
 plg 2
 plg 1
 plg 0.5
 plg 0.25
 plg 0.125
 plg 0.0625

exp. 2



plg 8
 plg 4
 plg 2
 plg 1
 plg 0.5
 plg 0.25
 plg 0.125
 plg 0.0625

exp. 3



plg 8
 plg 4
 plg 2
 plg 1
 plg 0.5
 plg 0.25
 plg 0.125
 plg 0.0625

Figure 5h

Plots of a versus plasminogen concentration
 $a = k_s \cdot k_{cat} \cdot PA \cdot E \cdot P / (K_m + P)$ The values were obtained by
 parabolic curve fitting to the equation $OD(t) = a \cdot t^2 + b \cdot t + c$

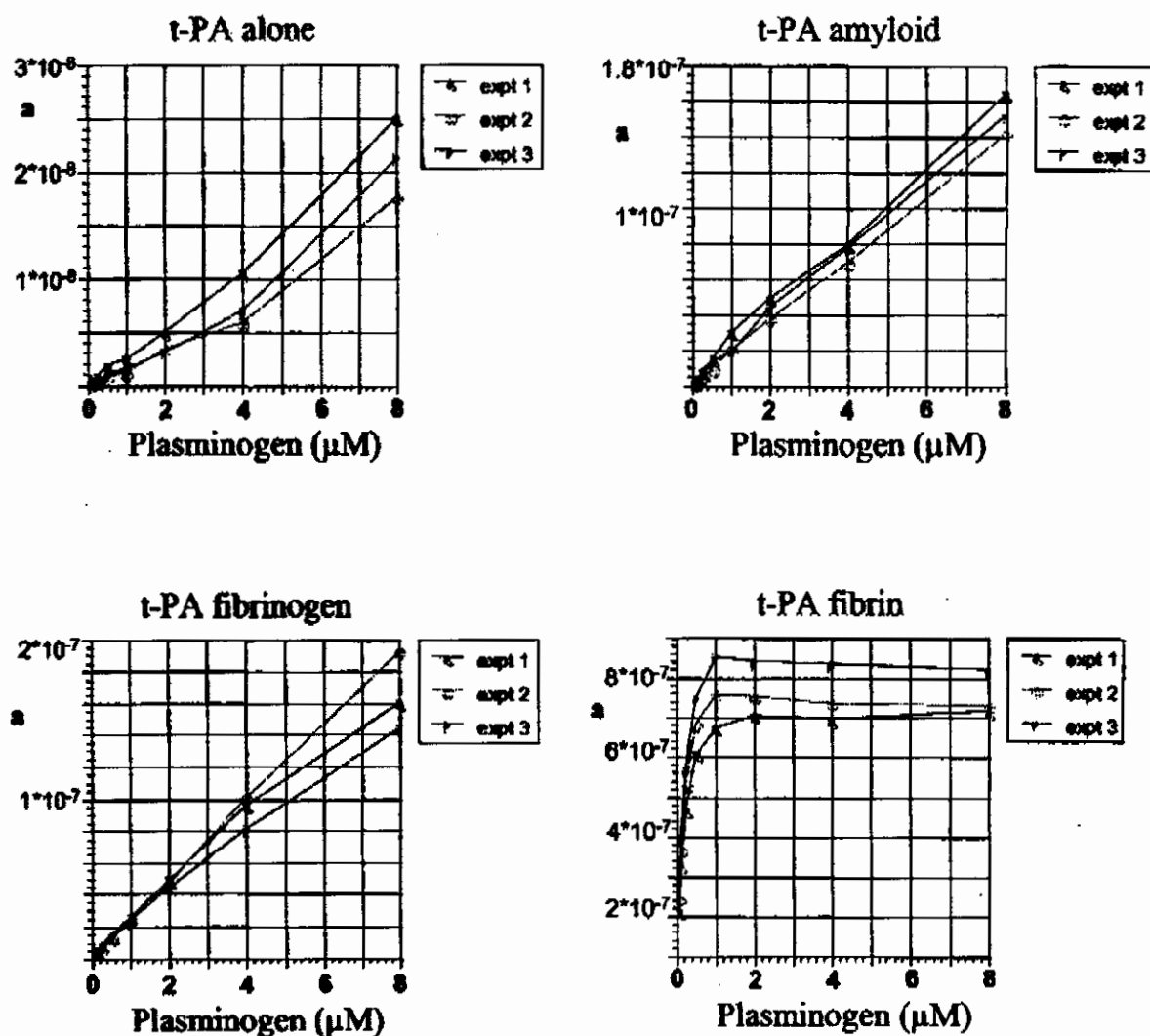


Figure 6a

Plots of a versus plasminogen concentration
 $a = k_s \cdot k_{cat} \cdot P \cdot A \cdot E \cdot P / (K_m + P)$ The values were obtained by
 parabolic curve fitting to the equation $OD(t) = a \cdot t^2 + b \cdot t + c$

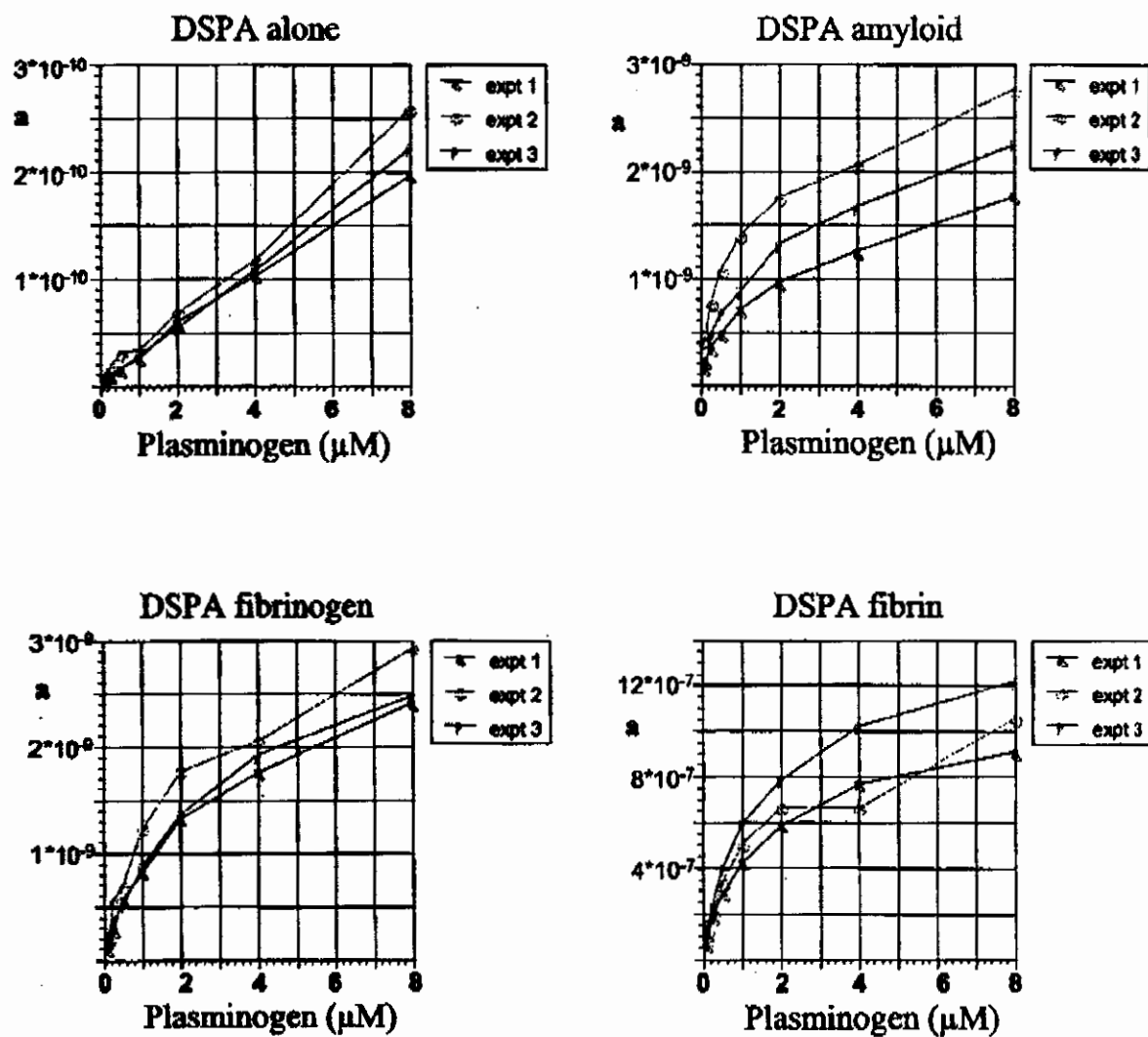


Figure 6b

Fig. 7

**Effect of PrP (#1) on tPA vs DSPA (comparison
with fibrin)**

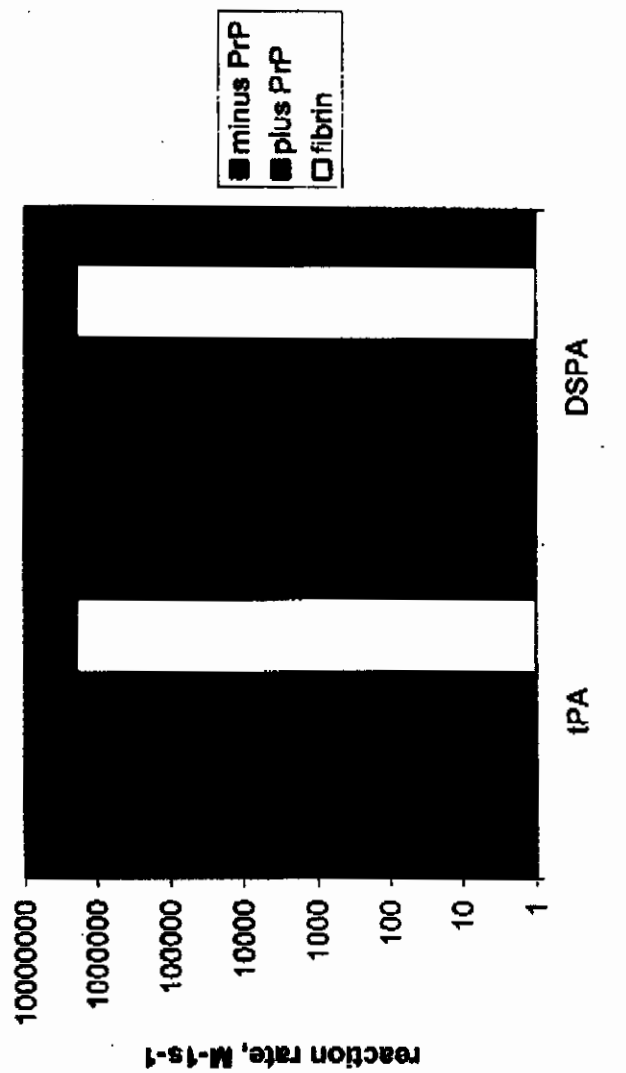


Fig. 8

**Effect of PrP (#1) on tPA vs DSPA (comparison
with fibrin)**

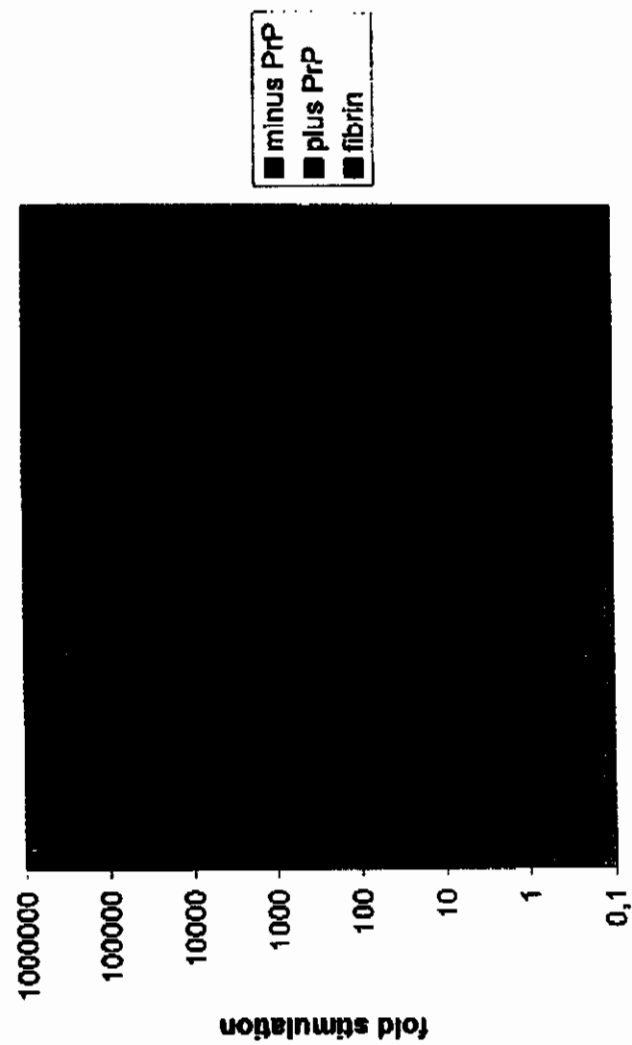


Fig. 9

Effect of PrP (#2) on tPA vs DSPA (including effect of heparin)

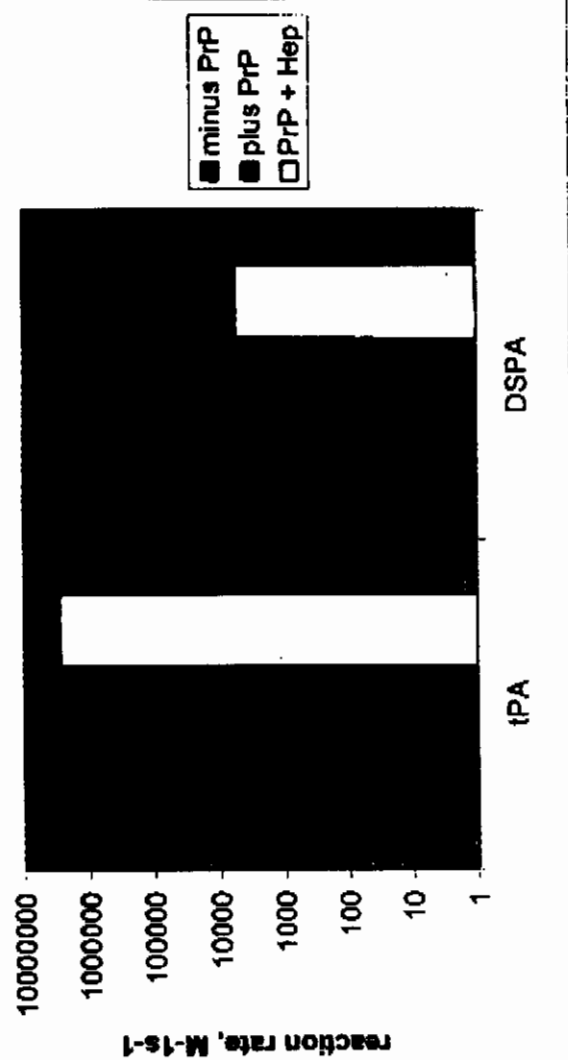


Fig. 10

**Effect of PrP (#2) on tPA vs DSPA (including
effect of heparin)**

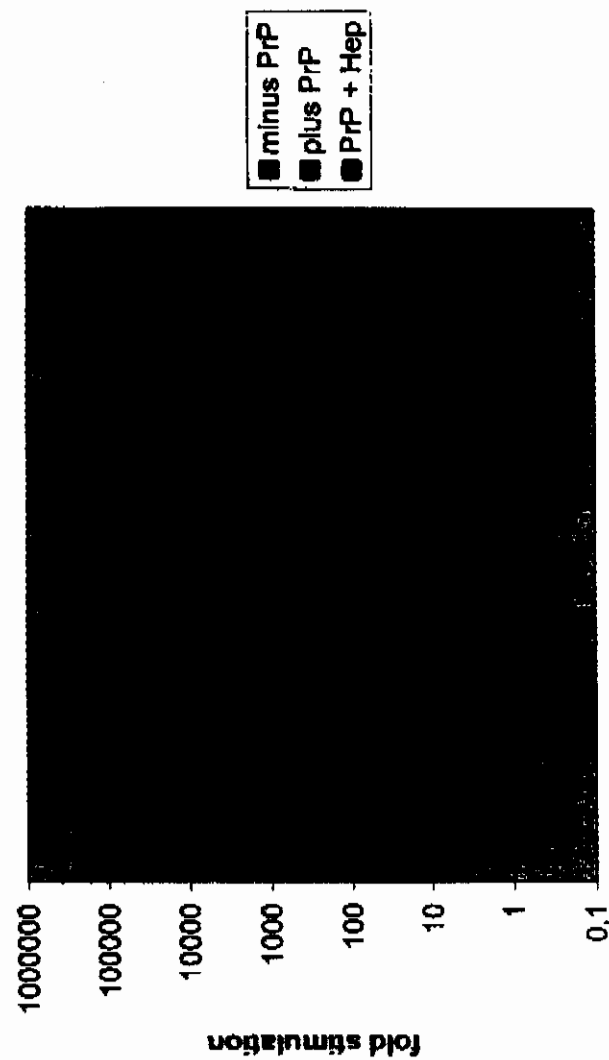


Fig. 11

MDAMKRG LCCVLLLCGAVFVSPSQEIHARFRRGARSYQVICRDEKTQMIYQQHQSWLRPVLRSNRVEY
CWCNSGRAQCCHSVPVKSCSEPRCTNGGTCQQALYFSDFVCQCPEGFAGKCCEIDTRATCYEDQGISYR
GTWSTAESGAECTNWNSSALAQKPYSGRRPDAILGLGNHNYCRNPDRDSKPWCYVFKAGKYSSEFCS
TPACSKATCGLRQYSQPQLHSTGGLFADIASHPWQAAIFAKHRRSPGERFLCGGILISSCWILSAHHC
FQERFPPHHLTVILGRTYRVVPGEEQKFEVEKYIVHKEFDDDTYNDIALQLKSOSSRCAQESSVV
RTVCLPPADLQLPDWTECELSGYGKHEALSPFYSERLKEAHVRLYPSSRCTSQHLLNRTVTDNMLCAG
DTRSGGPQANLHDACQGDGGPLVCLNDGRMTLVGII SWGLGCGQKDVPGVYTKVTNYLDWIRDNMRP

Fig. 12

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tPA      M-DAMKRGLCCVLLLCGAVFVSPSQEIHFRRGARSYQVICRDEKTQMIYQQHQSWLRP 59
Fig. 3   M-DAMKRGLCCVLLLCGAVFVSPSQEIHFRRGARSYQVICRDEKTQMIYQQHQSWLRP 59
Fig. 11  M-DAMKRGLCCVLLLCGAVFVSPSQEIHFRRGARSYQVICRDEKTQMIYQQHQSWLRP 59
DSPA_a1  MVNTMTKTKLLCVLLLCGAVFSLPRQETRYQLARGSRAYGVACKDEITQMTYRRQESWLRP 60
          : : ** * ***** * * * : : * : * * * : * * * * * : : : *****

tPA      VLRSNRVEYCWNSGRAQCHSVPVKSCSEPRCFNGGTCQQALYFSDFVCQCPEGFAGKCC 119
Fig. 3   VLRSNRVEYCWNSGRAQCHSVPVKSCSEPRCFNGGTCQQALYFSDFVCQCPEGFAGKCC 119
Fig. 11  VLRSNRVEYCWNSGRAQCHSVPVKSCSEPRCFNGGTCQQALYFSDFVCQCPEGFAGKCC 119
DSPA_a1  EVRSKRVEHCQCDRGOARCHTVPVNSCSEPRCFNGGTCWQAVYFSDFVCQCPAGYTGKRC 120
          : * : * : * * : * : * : * : * : * : * : * : * : * : * : * : *

tPA      EIDTRATCYEDQGISYRGTWSTAESGAECTNWNSSSALAQKPYSGRRPDAIRLGLGNHNYC 179
Fig. 3   EIDTRATCYEDQGISYRGTWSTAESGAECTNWNSSSALAQKPYSGRRPDAIRLGLGNHNYC 179
Fig. 11  EIDTRATCYEDQGISYRGTWSTAESGAECTNWNSSSALAQKPYSGRRPDAIRLGLGNHNYC 179
DSPA_a1  EVDTRATCYEGQGVTYRGTWSTAESRVECTNWNSSLLTRTYNGRMPDAFNLGLGNHNYC 180
          * : * : * : * : * : * : * : * : * : * : * : * : * : * : * : *

tPA      RNPDRDSKPWCYVFKAGKYSSEFCSTPACSEGNSDCYFGNGSAYRGTHSLTESGASCLPW 239
Fig. 3   RNPDRDSKPWCYVFKAGKYSSEFCSTPACSEGNSD----- 214
Fig. 11  RNPDRDSKPWCYVFKAGKYSSEFCSTPACSK----- 210
DSPA_a1  RNPNGAPKPWCYVIKAGKFTSESCSVPCSK----- 211
          *** : . * : * : * : * : * : * : * : * : * : * : * : * : * : * : * :

tPA      NSMILIGKVYTAQNPSAQALGLGKHNYCRNPDGDAKPWCHVLKNRRLTWBYCDVPSCSTC 299
Fig. 3   -----STC 217
Fig. 11  -----ATC 213
DSPA_a1  -----ATC 214
          : **

tPA      GLRQYSQPQFRIKGGFLPADIASHPWQAAIFAKHRRSPGERFLCGGILISSCWILSAAHCF 359
Fig. 3   GLRQYSQPOFHSGGFLPADIASHPWQAAIFAKHRRSPGERFLCGGILISSCWILSAAHCF 277
Fig. 11  GLRQYSQPQLHSTGGFLPADIASHPWQAAIFAKHRRSPGERFLCGGILISSCWILSAAHCF 273
DSPA_a1  GLRKYKEPQLHSTGGFLTDITSHPWQAAIFAQNRSSGERFLCGGILISSCWVLTAAHCF 274
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tPA      QERFPPHHLTIVILGRTYRVVPGEEBQKFEVEKYIVHKEFDDDTYDNDIALQLKSDSSRC 419
Fig. 3   QERFPPHHLTIVILGRTYRVVPGEEBQKFEVEKYIVHKEFDDDTYDNDIALQLKSDSSRC 337
Fig. 11  QERFPPHHLTIVILGRTYRVVPGEEBQKFEVEKYIVHKEFDDDTYDNDIALQLKSDSSRC 333
DSPA_a1  QESYLPDQLKVVLGRTYRVKPGEEBQTFKVKKYIVHKEFDDDTYNNDIALLQLKSDSPQC 334
          ** : * : * : * : * : * : * : * : * : * : * : * : * : * : * : * :

tPA      AQESSVVRTVCLPPADLQLPDWTECELSGYGKHEALSPFYSERLKEAHVRLYPSSRCTSQ 479
Fig. 3   AQESSVVRTVCLPPADLQLPDWTECELSGYGKHEALSPFYSERLKEAHVRLYPSSRCTSQ 397
Fig. 11  AQESSVVRTVCLPPADLQLPDWTECELSGYGKHEALSPFYSERLKEAHVRLYPSSRCTSQ 393
DSPA_a1  AQESDSVRAICLPEANLQLPDWTECELSGYGKHKSSSPFYSEQLKEGHVRLYPSSRCAPK 394
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tPA      HLLNRTVTDNMLCAGDTRSGGPQANLHDACQGDSSGGPLVCLNDGRMTLVGIISWGLGCGQ 539
Fig. 3   HLLNRTVTDNMLCAGDTRSGGPQANLHDACQGDSSGGPLVCLNDGRMTLVGIISWGLGCGQ 457
Fig. 11  HLLNRTVTDNMLCAGDTRSGGPQANLHDACQGDSSGGPLVCLNDGRMTLVGIISWGLGCGQ 453
DSPA_a1  FLFNKTVTNMLCAGDTRSGEIPNVHDACQGDSSGGPLVCMNDNHMTLLGIISWGVGCGE 454
          . * : * : * : * : * : * : * : * : * : * : * : * : * : * : * : * :

tPA      KDVPGVYTKVTNYLDWIRDNMRP 562
Fig. 3   KDVPGVYTKVTNYLDWIRDNMRP 480
Fig. 11  KDVPGVYTKVTNYLDWIRDNMRP 476
DSPA_a1  KDVPGVYTKVTNYLGWIRDNMHL 477

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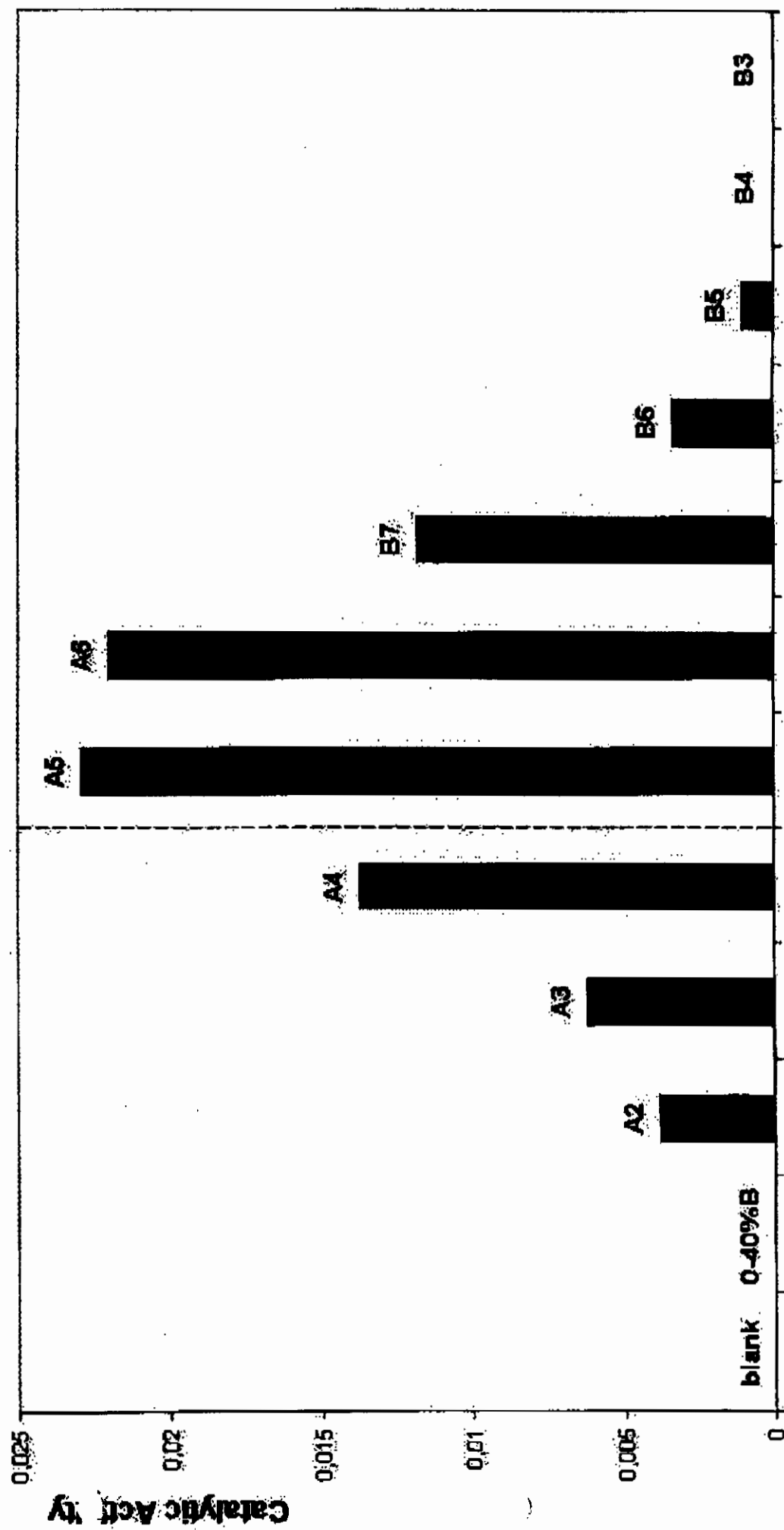
→ tPa
 → word tPa (Fig 2)
 → word tPa (Fig 3)
 → DSPa

Fig. 13

MDAMKRG LCCVLLLCGAVFSLPSQETHRQLARGSRAYQVACRDEITQMIYQQHQSWLRPVLRSKRVEH
CQCNRGQARCHSVFVKSCSEPRCFNGGTCWQALYPSDFVCQCPEGYTGKCCEIDTRATCYEGOGISYR
GTWSTAESRVECTNWNSSLLTRRTYNGRMPDAFNLGLGNHNYCRNPNGAPKPWCYVIKAGKFSSES
VPACSKATCGLRKYKEPQLHSTGGLEFTDIASHPWQAAIFAQHRRSSGERFLCGGILISSCWILSAAHC
FQESYLPDQLKVVLGRTYRVVPGEETQTFKVKYIVHKEFDDDTYDNDIALQLKSDSPQCAQESDSV
RAICLPEANLQLPDWTECELSGYGKHESSEPFYSEQLKEGHVRLYPSSRCAPKFLFNRTVTNNMLCAG
DTRSGEYIPNVHDACQGDSSGPLVCMNDNHMTLVGTIISWGVGCGQKDVPQVYTKVTNYLGWIRDNMHL

Fig. 14

S-2288 Activity of vsmptPA-SPXL-Column Fractions



Silver-stained SDS-PAGE Gel of SPXL-column fractions

Fig. 15 a

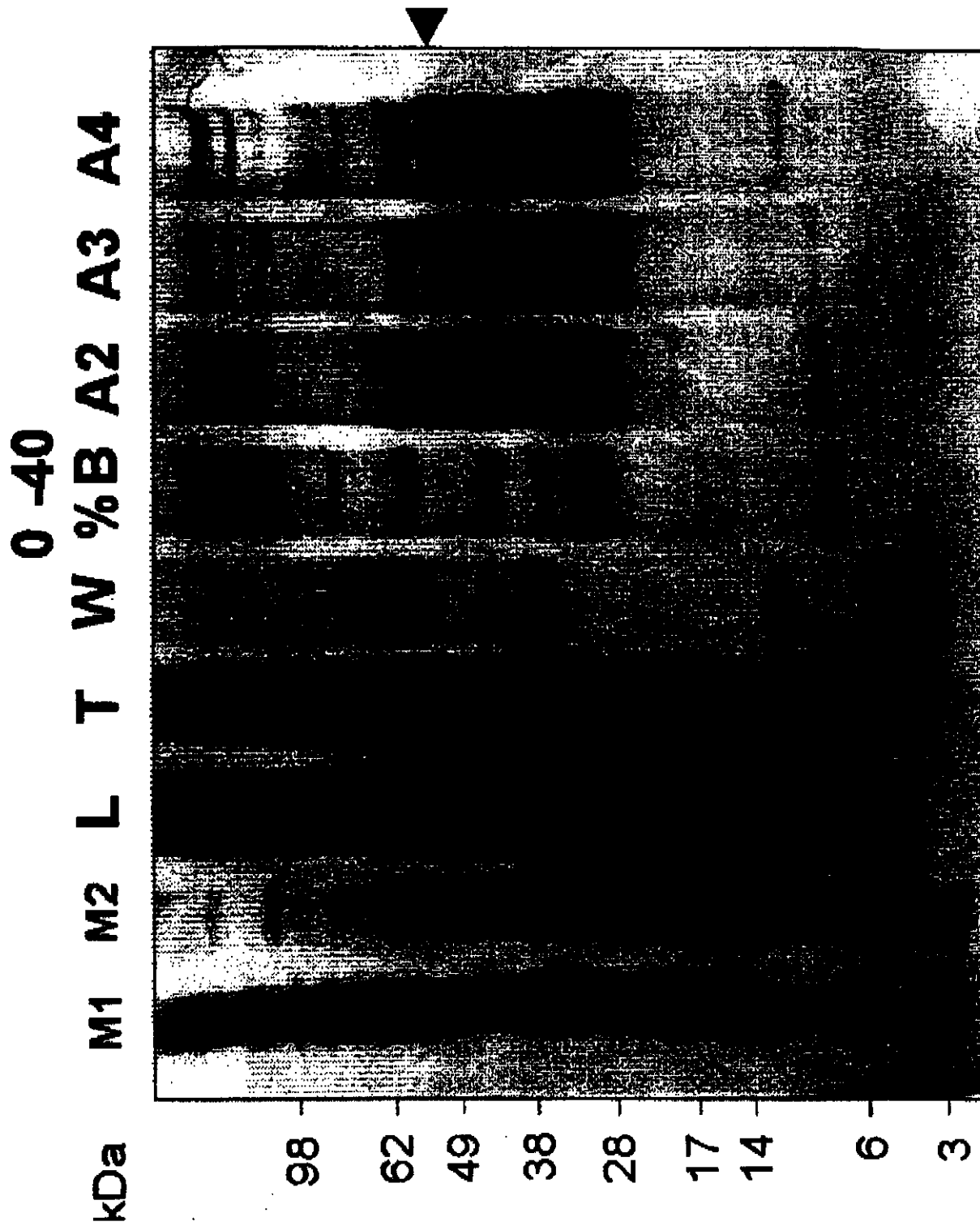


Fig. 15 b

Silver-stained SDS-PAGE Gel of SPXL-column fractions

kDa M1 M2 A5 A6 B7 B6 B5 B4

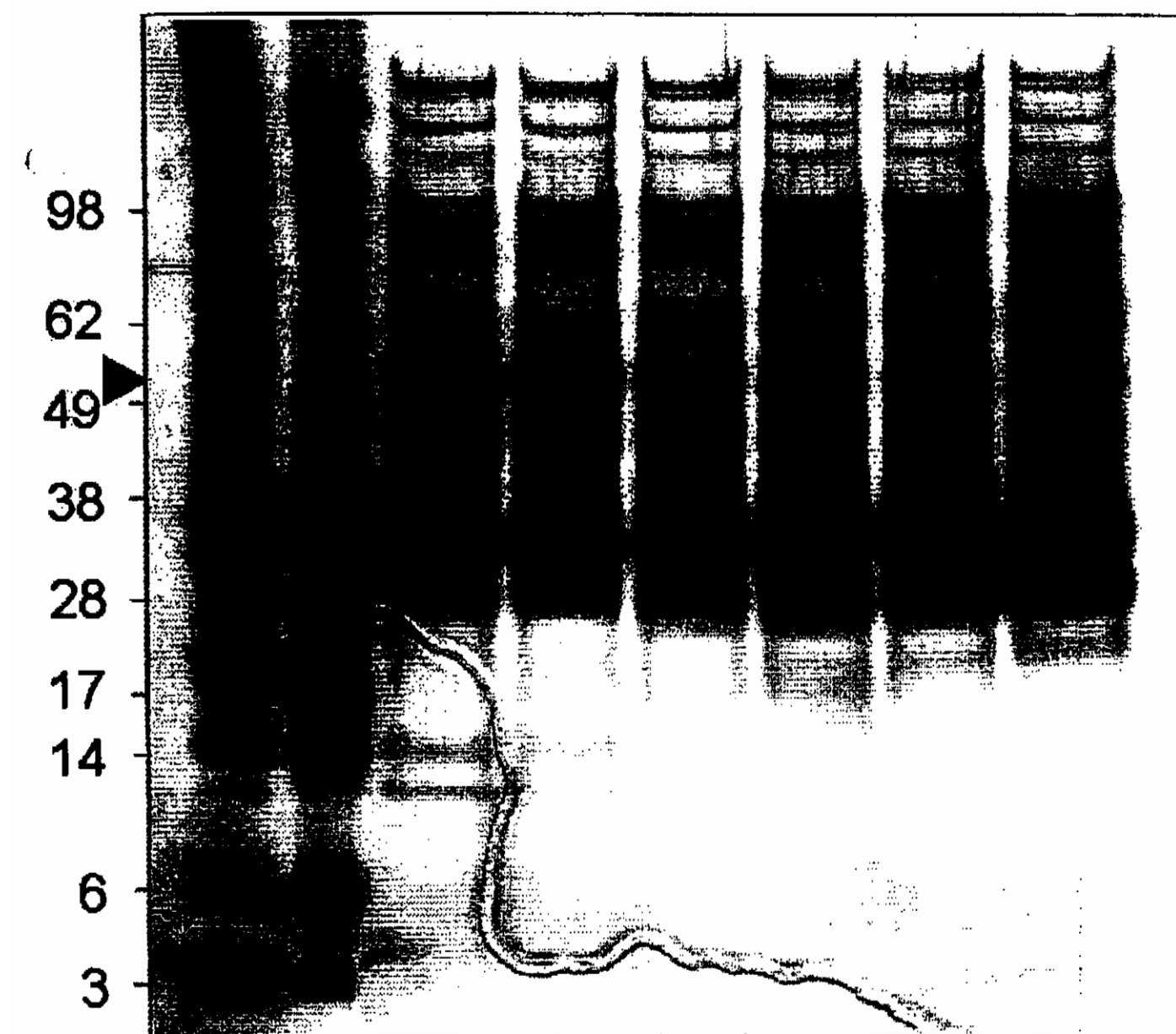
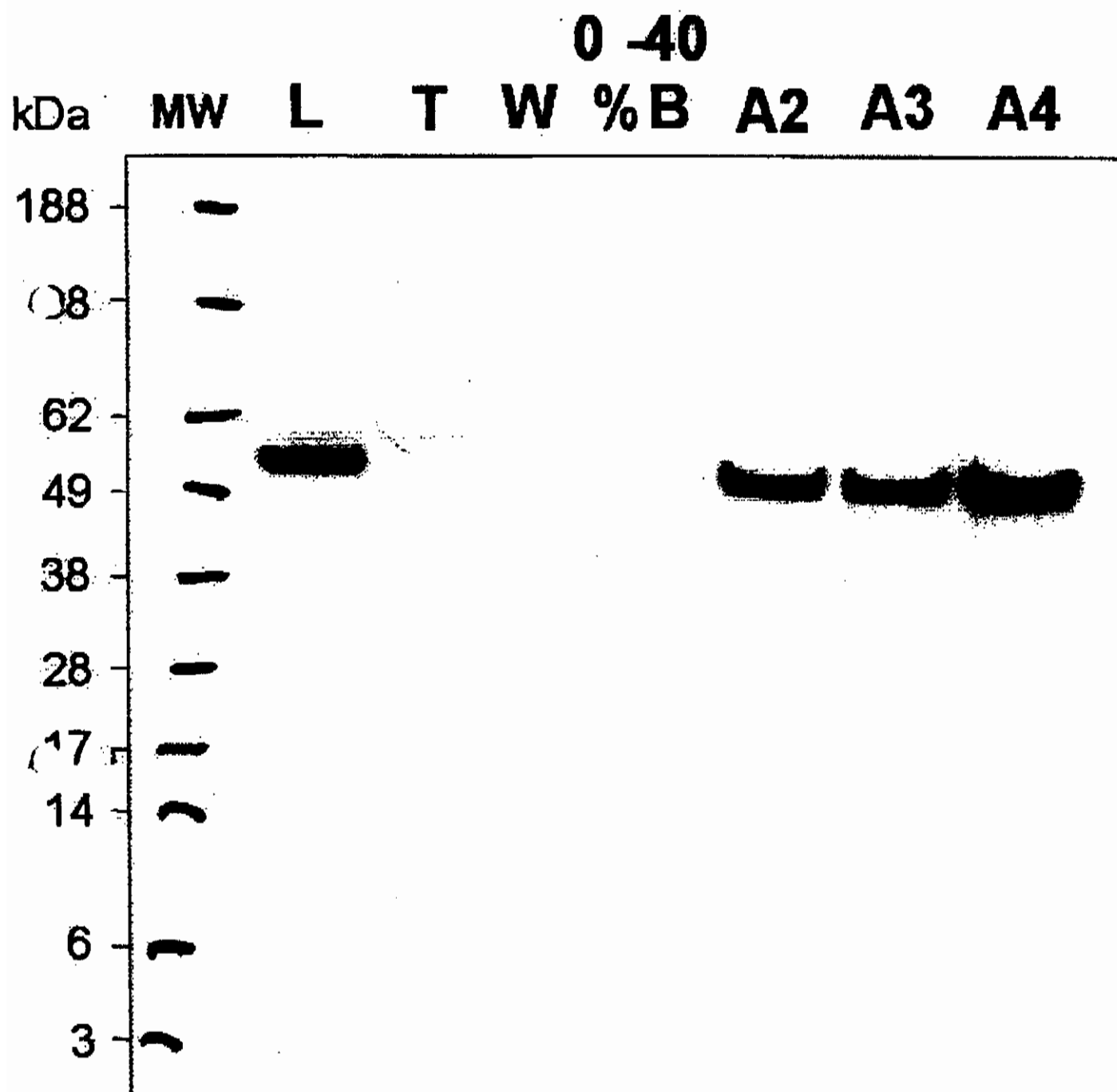


Fig. 16 a

Western Blot of SPXL-column fractions



Western Blot of SPXL-column fractions

Fig. 16 b

kDa MW A5 A6 B7 B6 B5 B4

