

Fig. 1

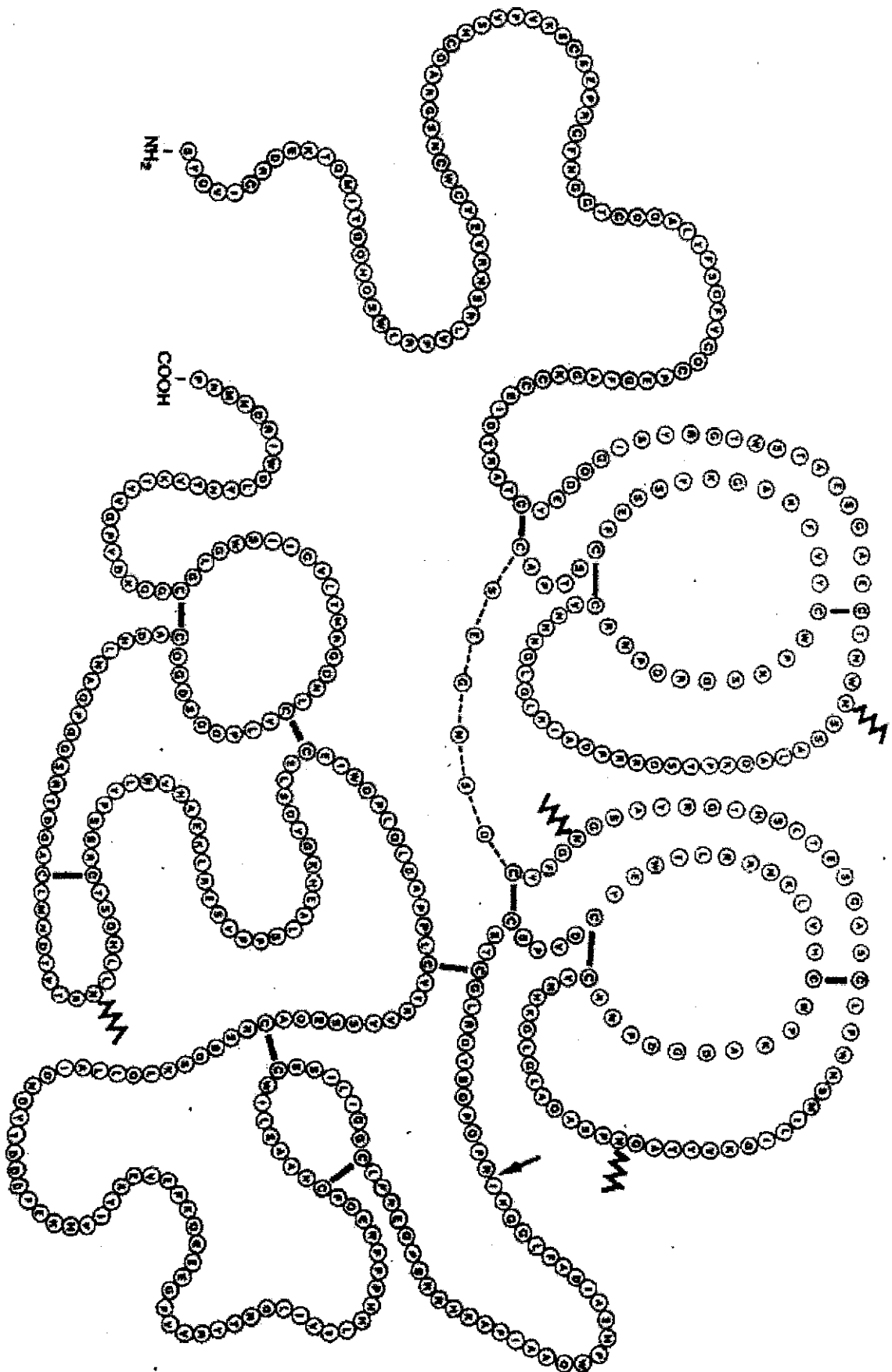


Fig. 2

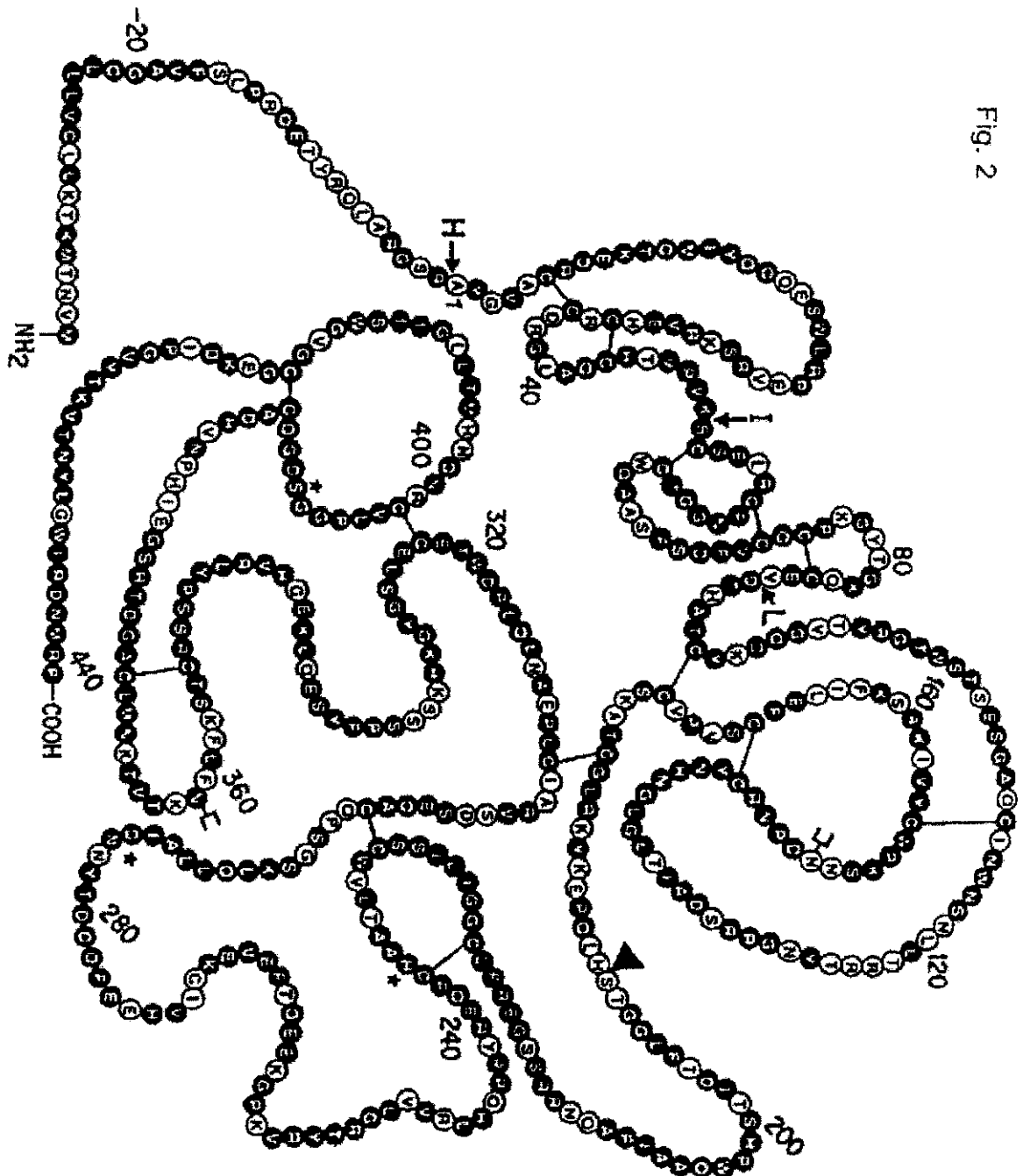


Fig. 2b

[GENETYX-MAC: Multiple Alignment]
Date : 2003.08.23

tPA Isoform 1 vs DSPA alpha 1

tPA_AS	1	M-DAMKRGLCCVLLLCGAVFVSPSQEIHARFRRGARSYQVICRDEKTQMIYQQHQSWLRP	59
DSPA_AS	1	MVNTMKTKLLCVLLLCGAVFSLPRQETRYQLARGSRAYGVACKDEITQMTYRRQESWLRP	60
		* ** * ***** * ** * * * * * * * * * * *	
tPA_AS	60	VLRNVRVEYCWNSGRAQCHSVPVKSCSEPRCFNGGTCQQALYFSDFVCQCPEGFAGKCC	119
DSPA_AS	61	EVRSKRVEHCQCDRGQARCHTVPVNSCSEPRCFNGGTCWQAVYFSDFVCQCPAGYTGKRC	120
		** *	
tPA_AS	120	EIDTRATCYEDQGISYRGTWSTAESGAECTNWNSSALAQKPYSGRRPDAILRLGLGNHNYC	179
DSPA_AS	121	EVDTRATCYEGQGVTYRGTWSTAESRVEICINWNSSLTRRTYNGRMPDAFNLGLGNHNYC	180
		* *	
tPA_AS	180	RNPDRDSKPWCYVFKAGKYSSEFCSTPACSEGNSDCYFGNGSAYRGTHSLTESGASCLPW	239
DSPA_AS	181	RNPNGAPKPWCYVIKAGKFTSESCSVPCSK-----	204
		*** * * * * * *	
tPA_AS	240	NSMILIGKVYTAQNPSAQAALGLGKHNYCRNPDGDAKPWCHVLKNRRLTWEYCDVPSCSTC	299
DSPA_AS	205	-----ATC	214
		**	
tPA_AS	300	GLRQYSQPQFRIKGGLFADIASHPWQAAIFAKHRRSPGERFLCGGILISSCWILSAAHCF	359
DSPA_AS	215	GLRKYKEPQLHSTGGLFTDITSHPWQAAIFAQNRSSGERFLCGGILISSCWVLTAAHCF	274
		*** *	
tPA_AS	360	QERFPPHHLTVILGRTYRVVPGEQKFEVEKYIVHKEFDDDTYDNDIALQLKSDSSRC	419
DSPA_AS	275	QESYLPDQLKVVLGRTYRVKPGEEQTFKVKKYIVHKEFDDDTYNNDIALQLKSDSPQC	334
		** *	
tPA_AS	420	AQESSVVRTVCLPPADLQLPDWTECELSGYGKHEALSPFYSERLKEAHVRLYPSSRCTSQ	479
DSPA_AS	335	AQESDSVRAICLPEANLQLPDWTECELSGYGKHKSSSPFYSEQLKEGHVRLYPSSRCAPK	394
		**** *	
tPA_AS	480	HLLNRTVTDNMLCAGDTRSGGPQANLHDACQGDGGPLVCLNDGRMTLVGIISWGLGCGQ	539
DSPA_AS	395	FLFNKTVTNMLCAGDTRSGEIYPNVHDACQGDGGPLVCMNDNHMTLLGIISWGVGCGE	454
		* *	
tPA_AS	540	KDVPGVYTKVTNYLDWIRDNMRP	562
DSPA_AS	455	KDVPGVYTKVTNYLGWIRDNMHL	477

Kringel 2

tPA Activation Site

Fig. 3**Humanised DSPA**

Sequence is based on tPA Isoform 1 with

- a) Deletion of Kringel 2
- b) tPA activation site mutated to corresponding DSPA sequence

```

1 mdamkrglcc vlllcgavfv spsqeiharf rrgarsyqvi crdektqmiy qqhqswlrpv
61 lrsnrveycw cnsgraqchs vpvkscsepr cfnggtcqqa lyfsdfvcqc pegfagkcce
121 idtratcyed qgisyrqtws taesgaectn wnssalaqkp ysgrrpdair lglgnhnycr
181 npdrdskpw cyvfkgky ssefcstpa csegnsdst cglrqysqp qfhskgglf
241 adiaspww aaifakhrr spgerflcg gilisscw lsaahcfqe rfpphhltv
301 ilgtryrvv pgeeeqkfe vekiivhke fdddynd iallqlksd ssrcaqess
361 vrvtvcclpp adlqlpdwt ecelsgygk healpfys erlkeahvr lypssrcts
421 qhllnrtvt dnmlcagdt rsggpqanl hdacqgdsg gplvcldng rmtlvgiis
481 wglgcgqkd vpgvytkvt nyldwirdn mrp

```

Italics: Signal sequence

Kringel 2 Deletion: nsd-Kringel2-stc

mutated tPA Activation Site according to DSPA

Conversion of OD405 into converted substrate

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

$$R^2 = 0.9996$$

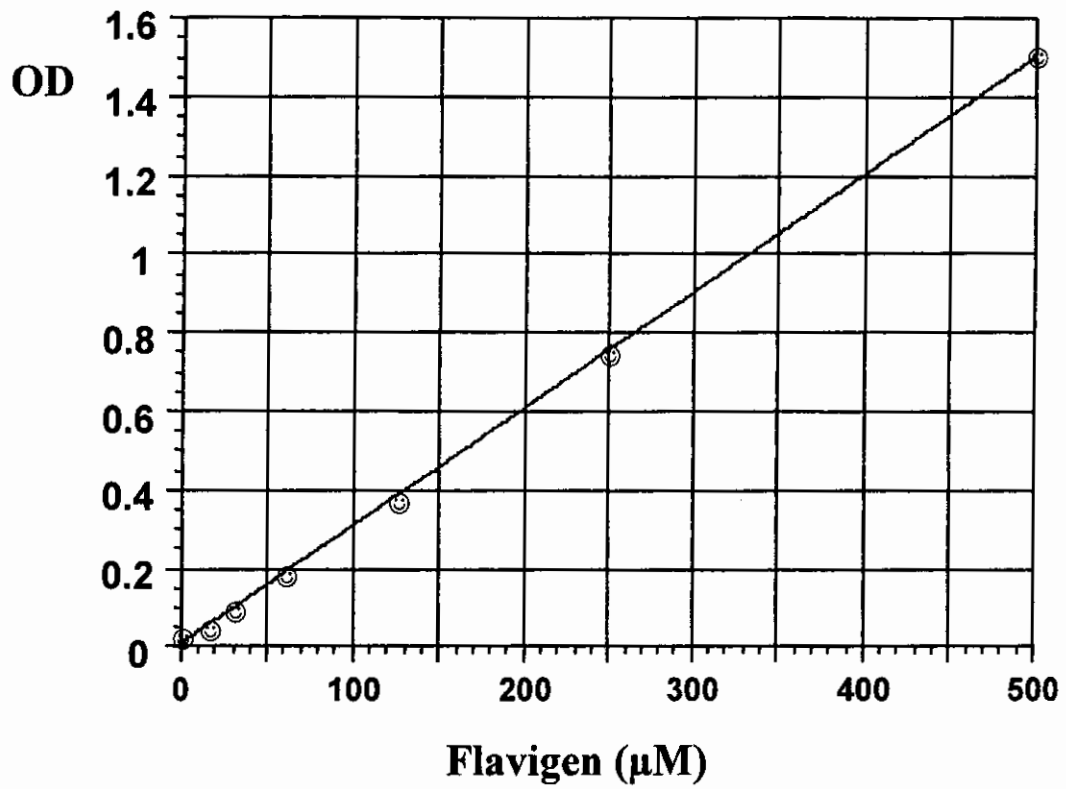


Figure 4a

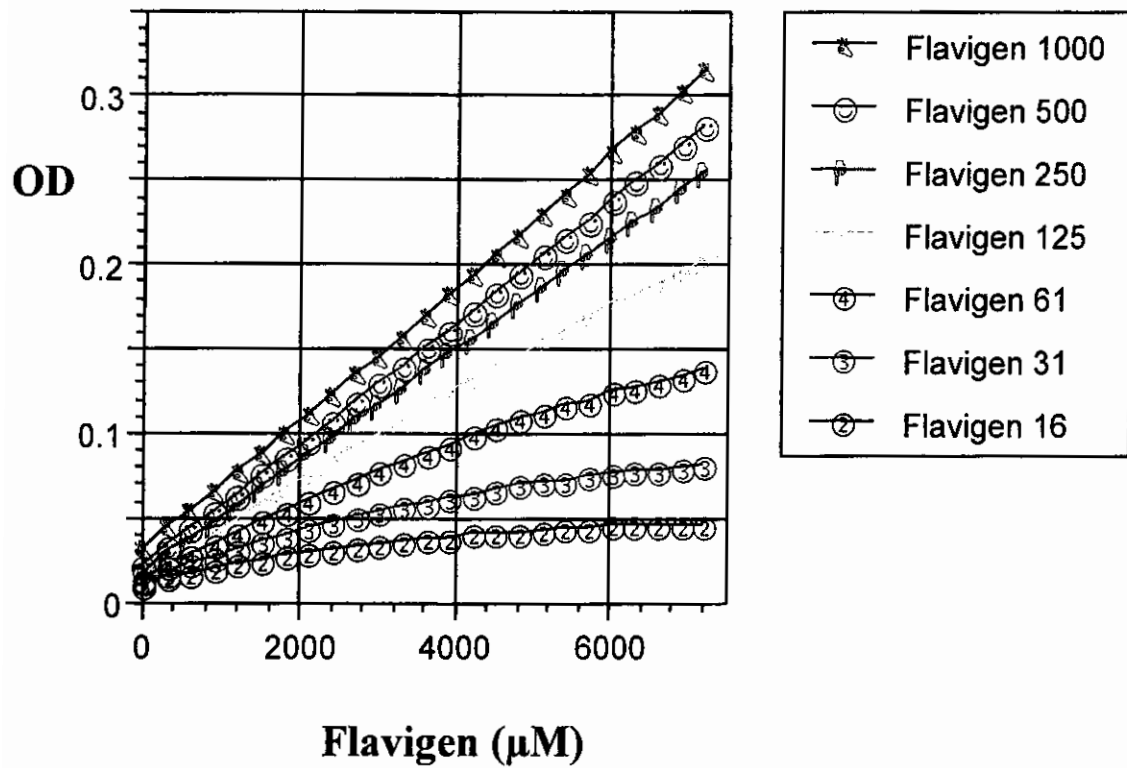
Conversion of Flavigen.pli by 10 nM plasmin

Figure 4b

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

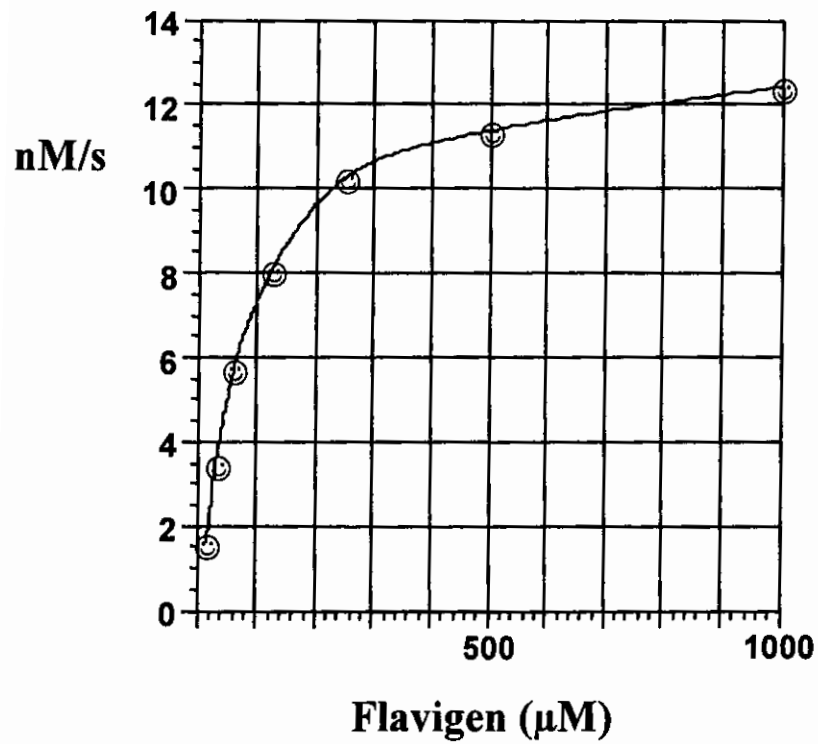
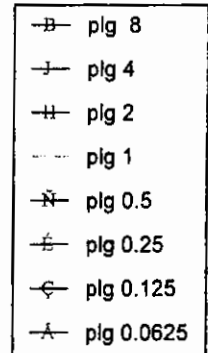
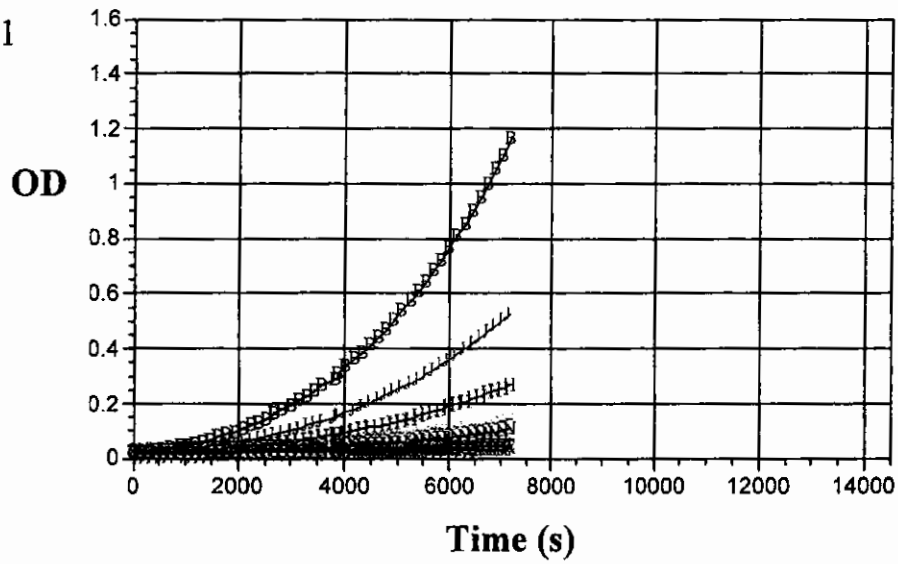


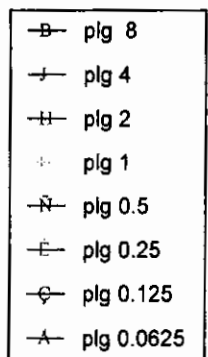
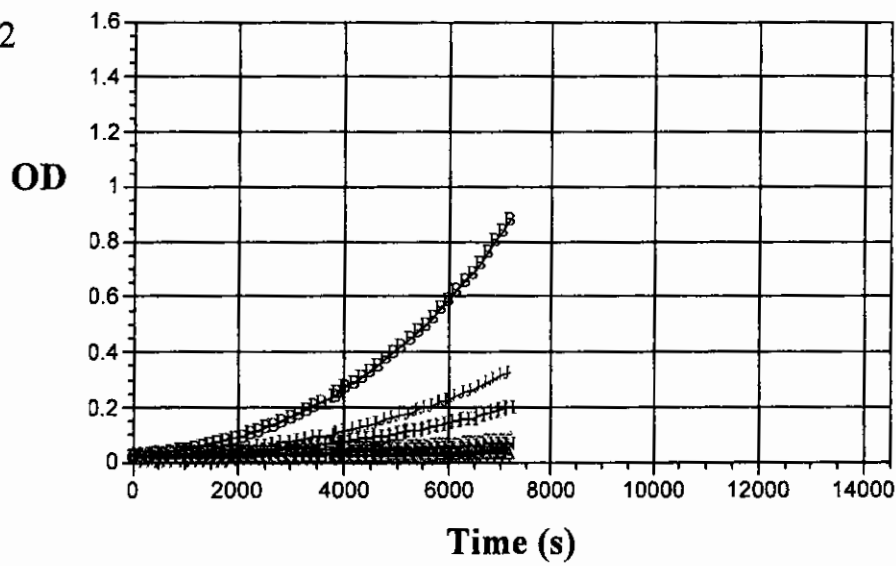
Figure 4c

t-PA alone no cofactor

exp. 1



exp. 2



exp. 3

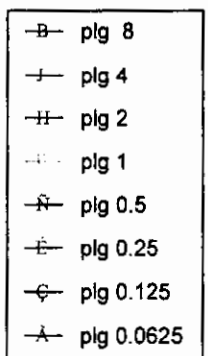
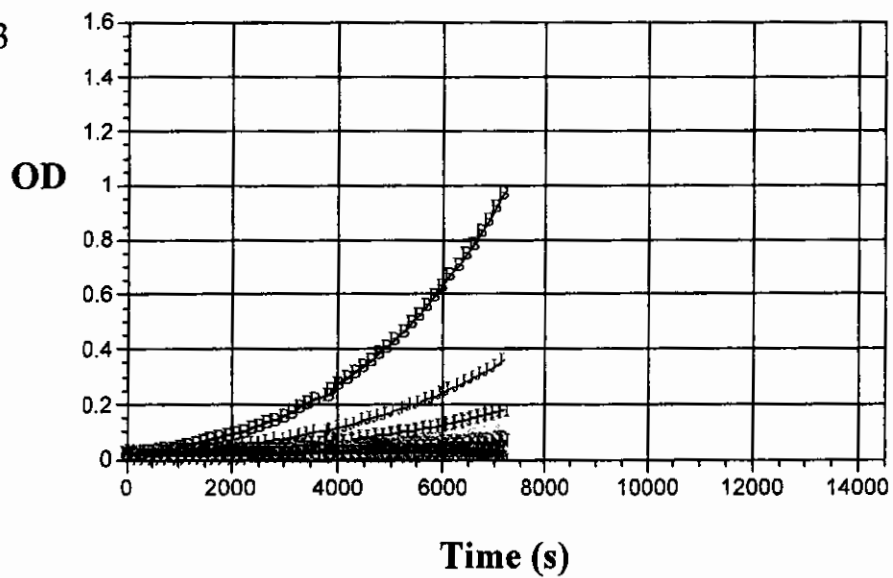
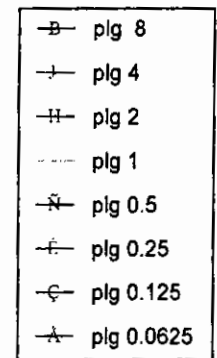
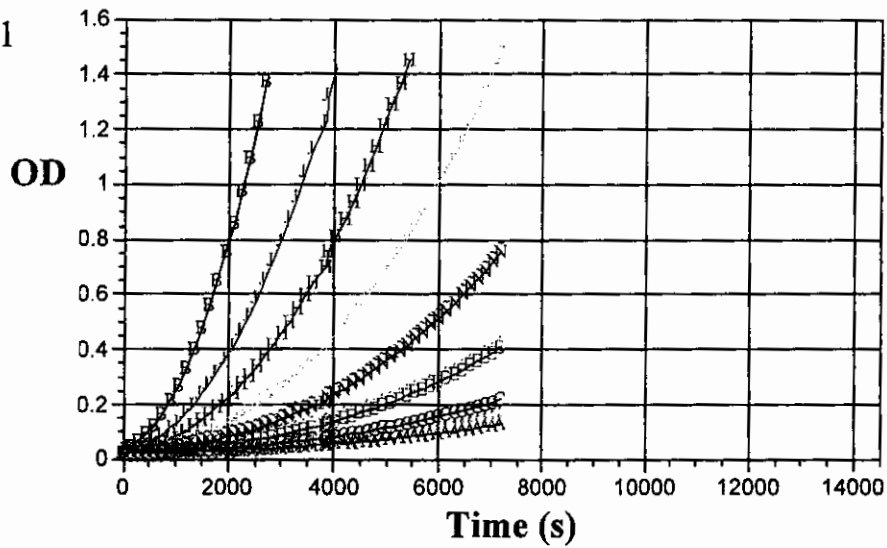


Figure 5a

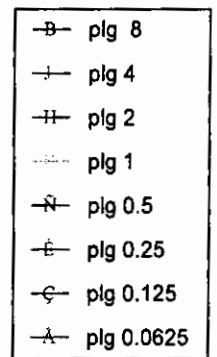
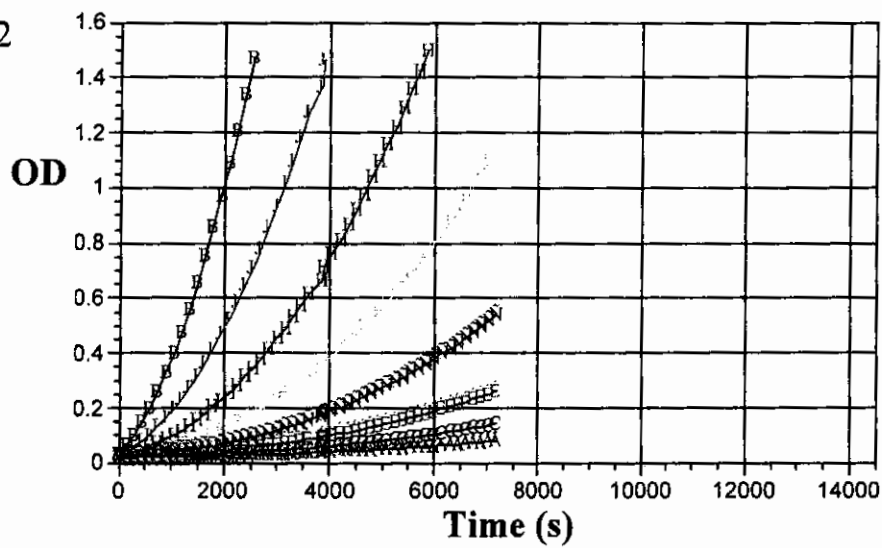
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t-PA , b-amyloid

exp. 1



exp. 2



exp. 3

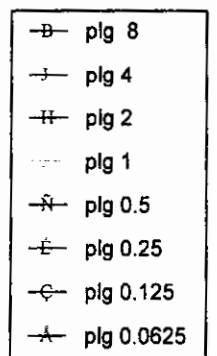
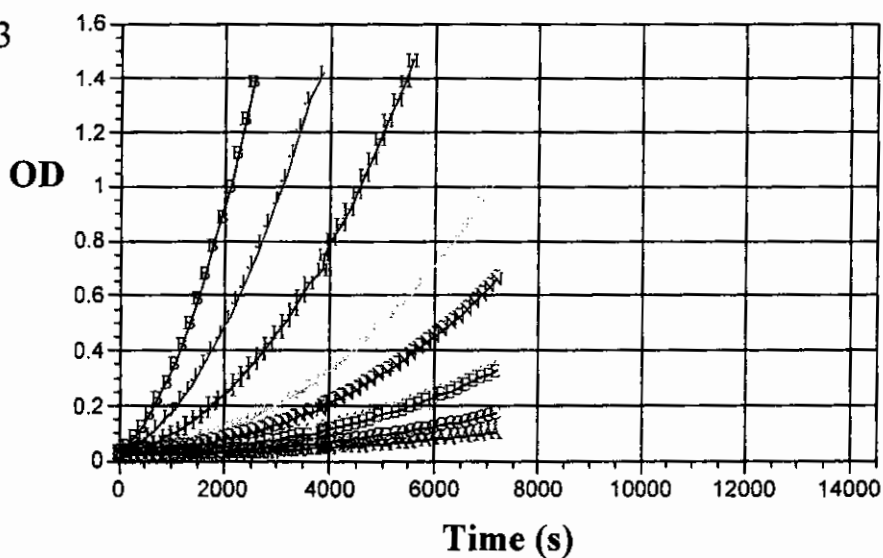
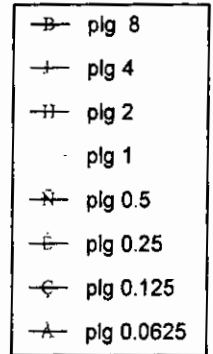
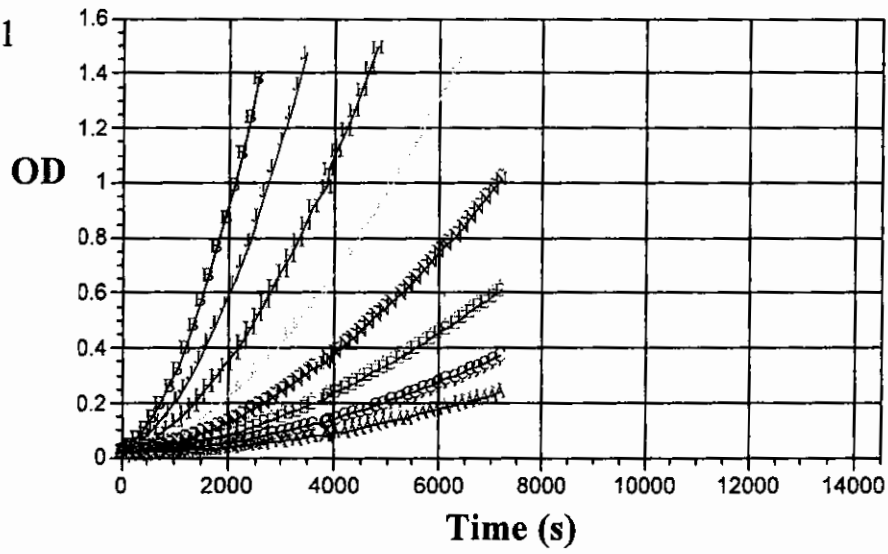


Figure 5b

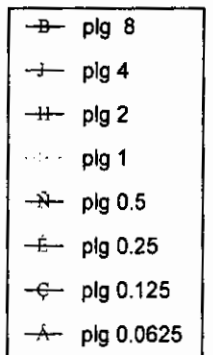
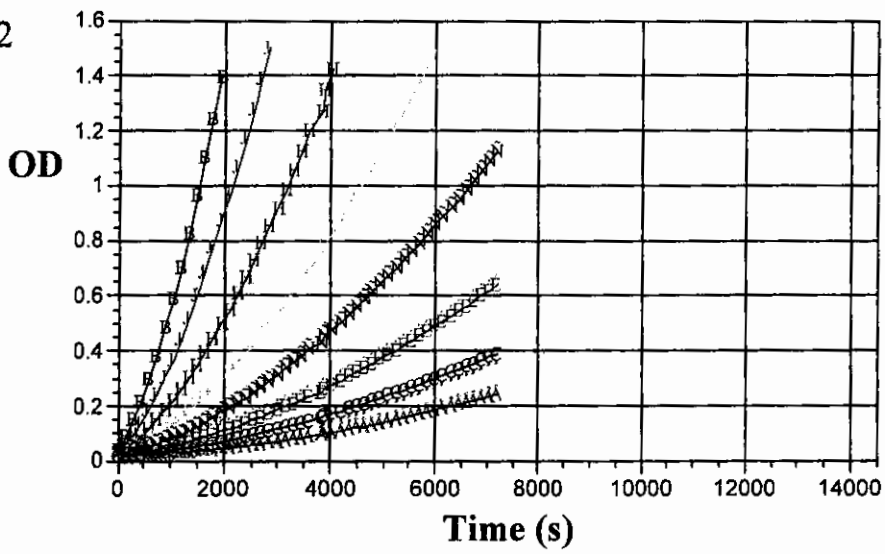
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t-PA , fibrinogen

exp. 1



exp. 2



exp. 3

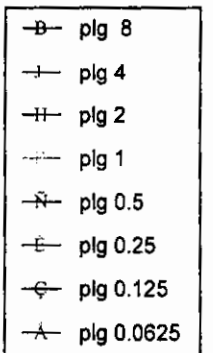
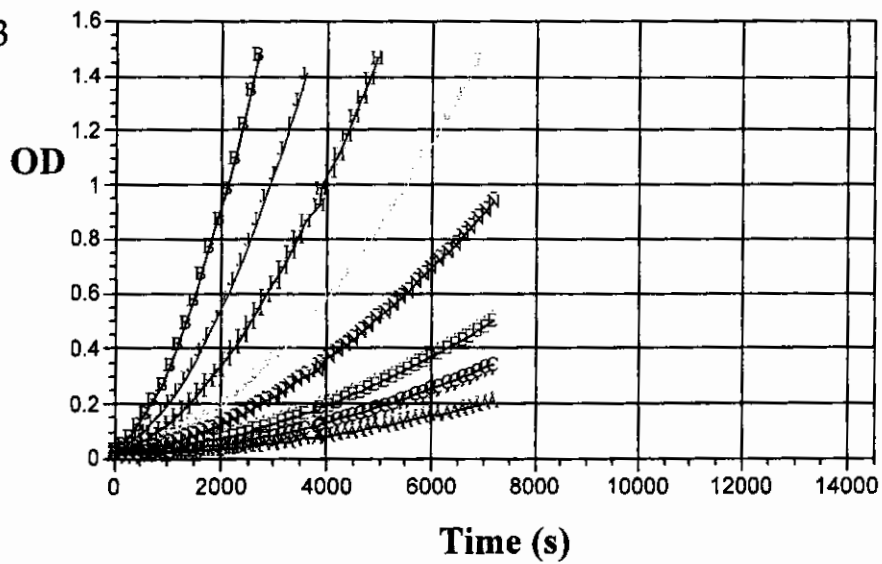
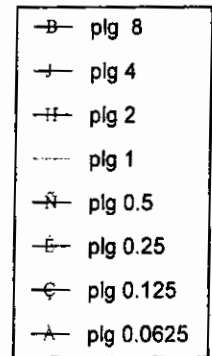
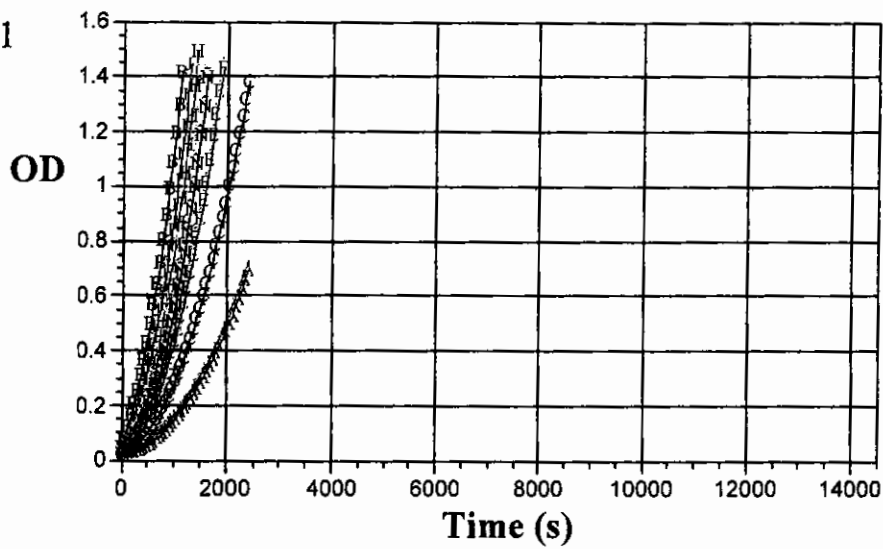


Figure 5c

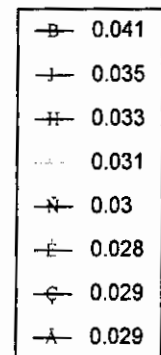
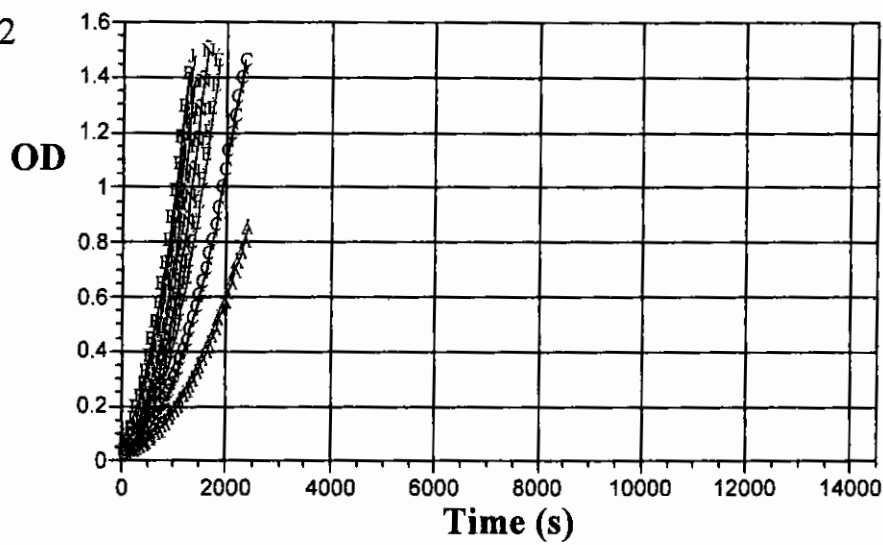
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t-PA, fibrin

exp. 1



exp. 2



exp. 3

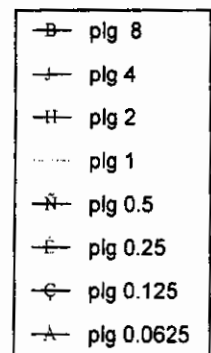
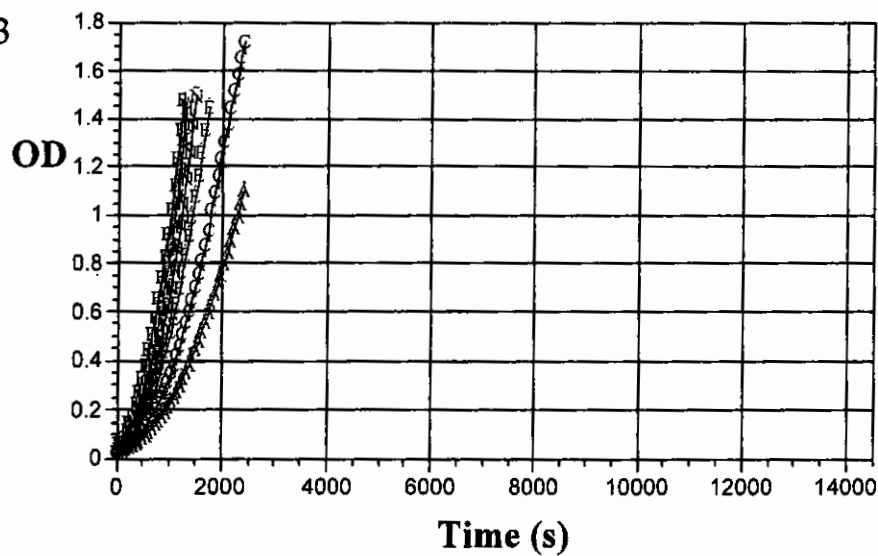
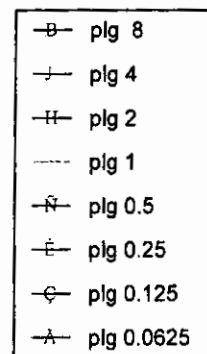
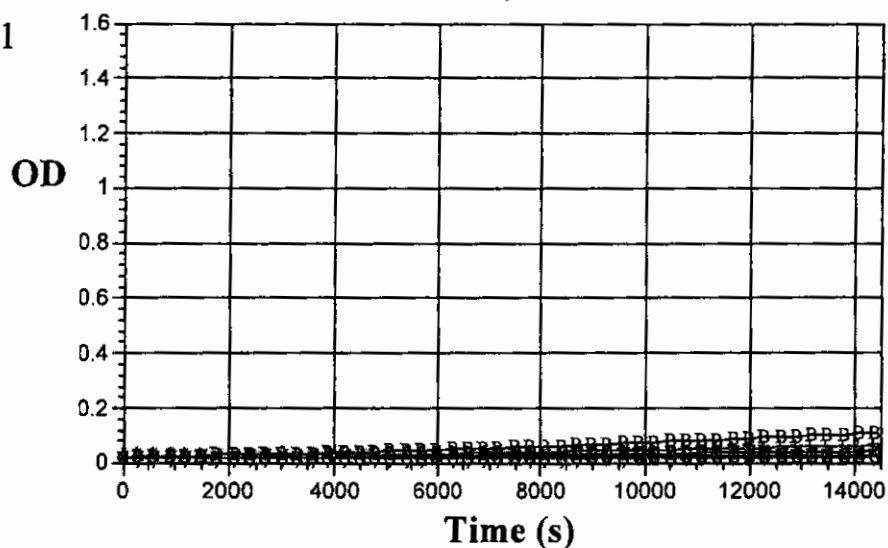


Figure 5d

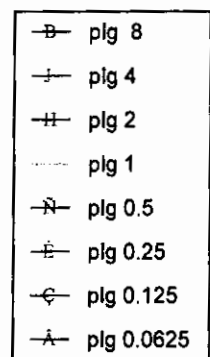
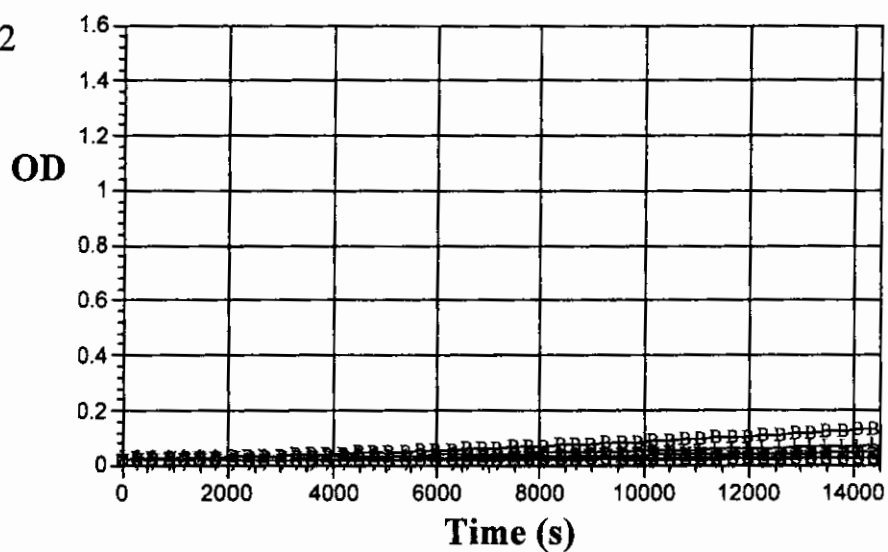
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DSPA, no cofactor

exp. 1



exp. 2



exp. 3

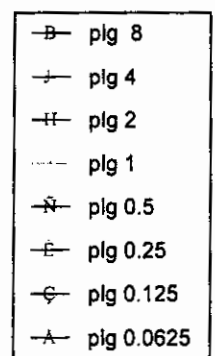
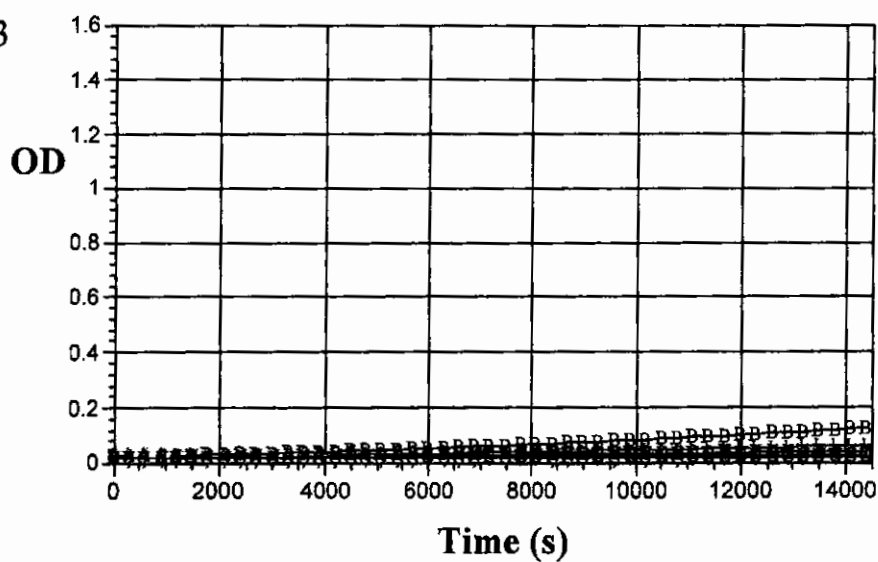
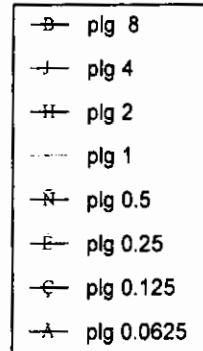
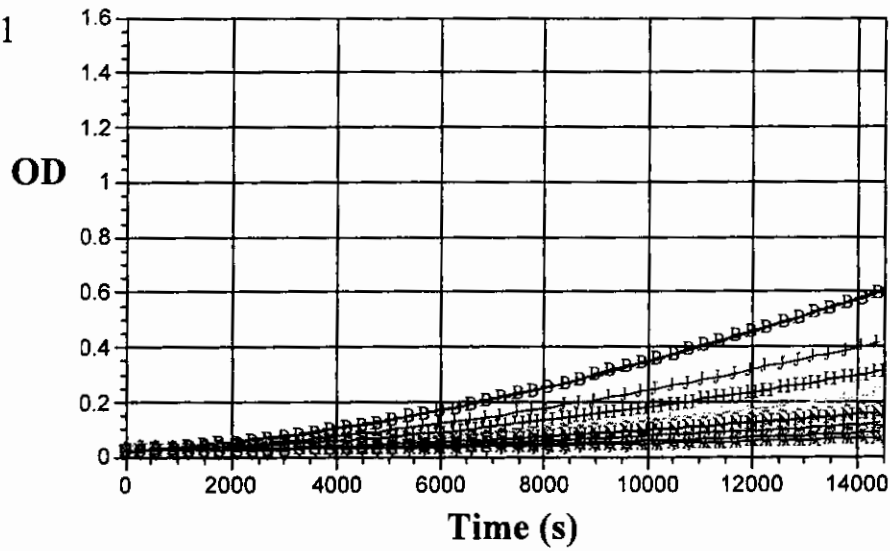


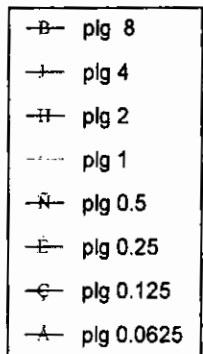
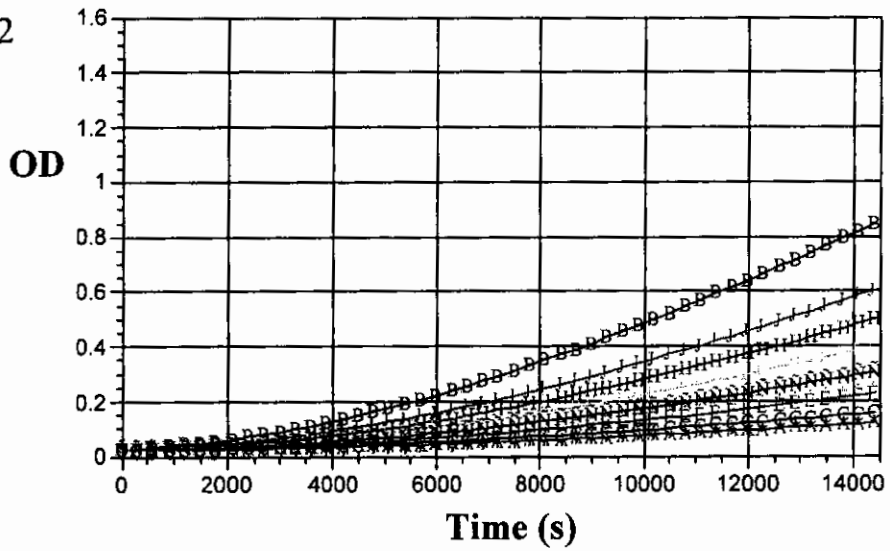
Figure 5e

DSPA, b-amyloid

exp. 1



exp. 2



exp. 3

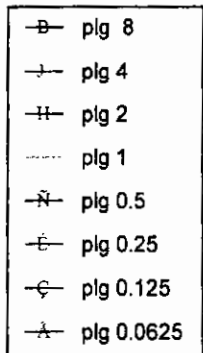
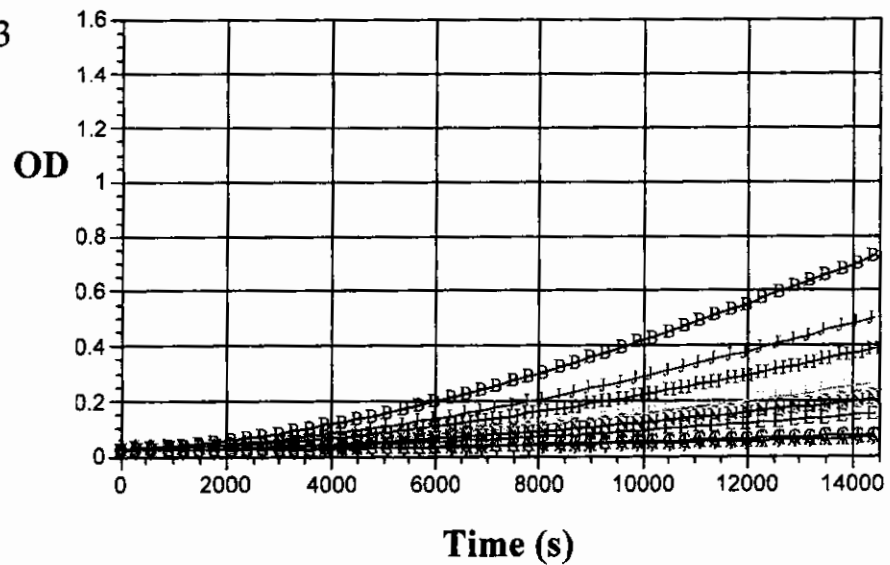
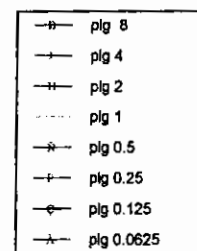
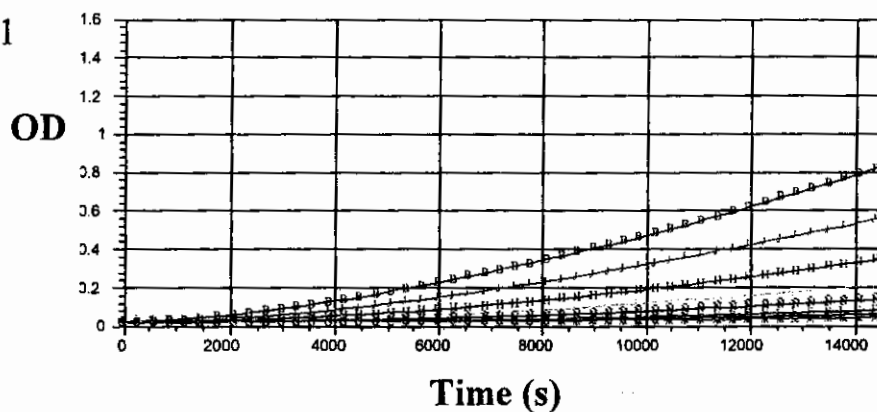


Figure 5f

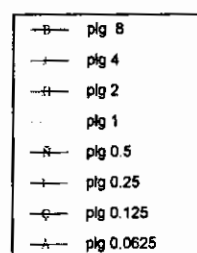
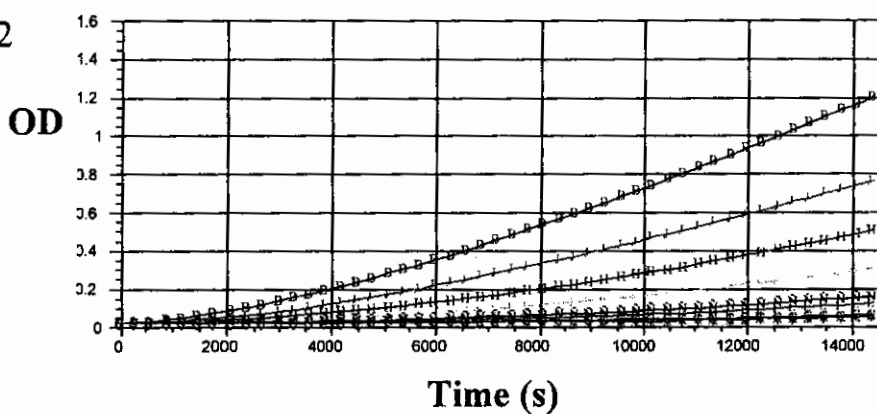
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DSPA, fibrinogen

exp. 1



exp. 2



exp. 3

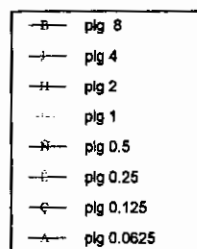
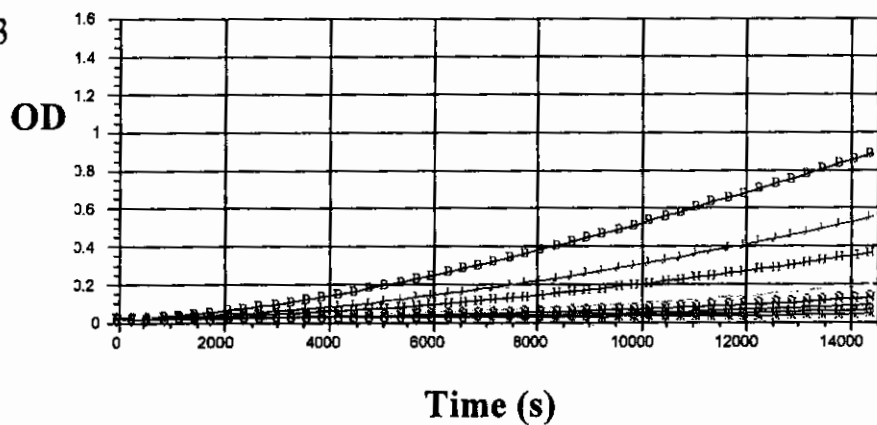
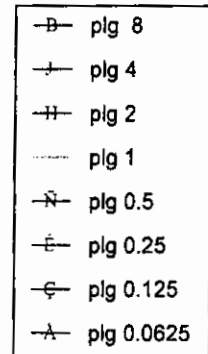
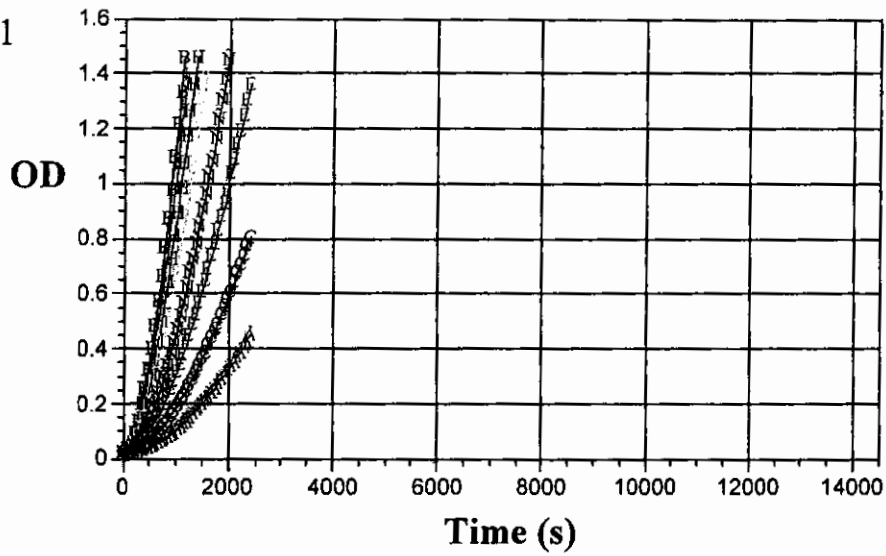


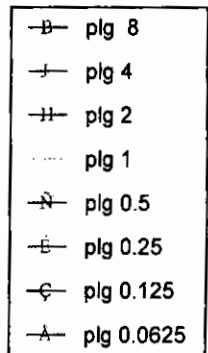
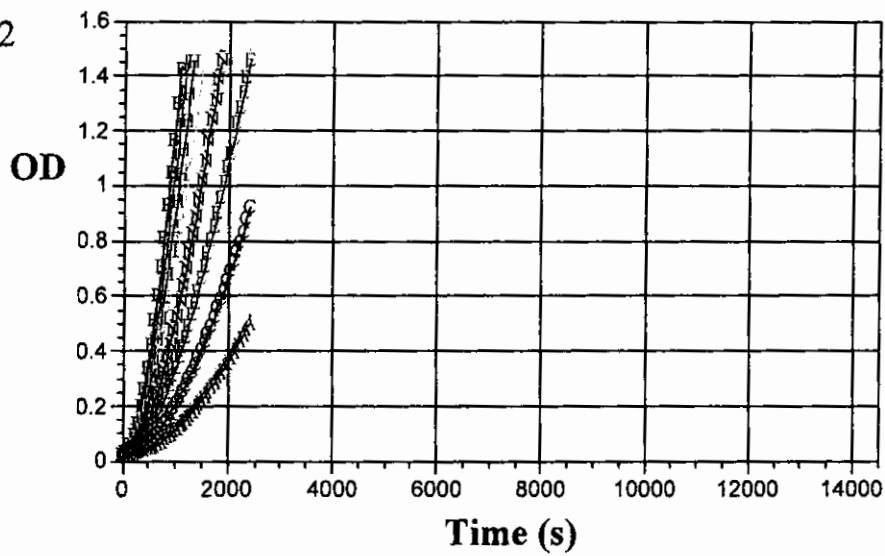
Figure 5g

DSPA , fibrin

exp. 1



exp. 2



exp. 3

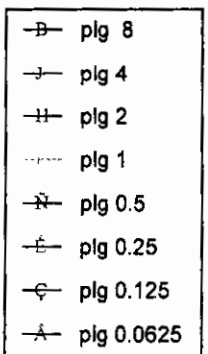
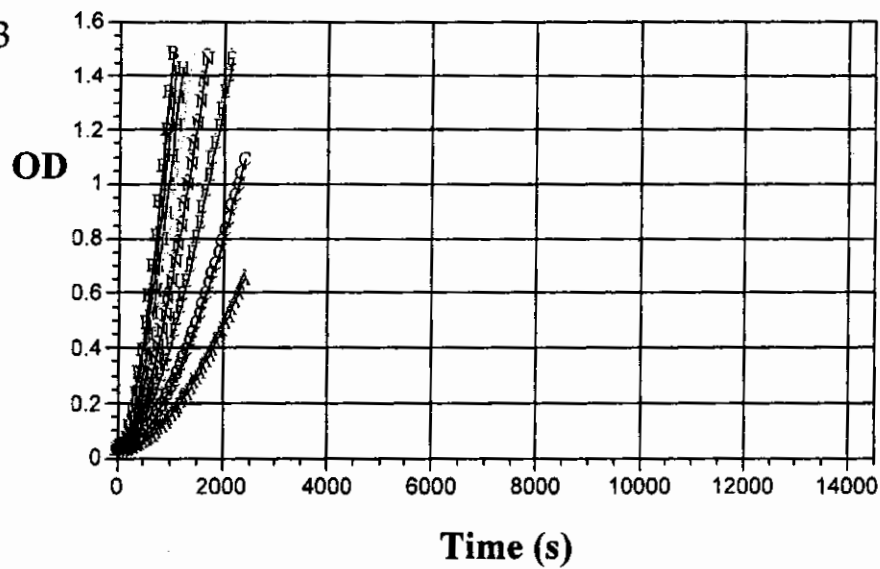


Figure 5h

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 $OD(t) = a \cdot t^2 + b \cdot t + c$

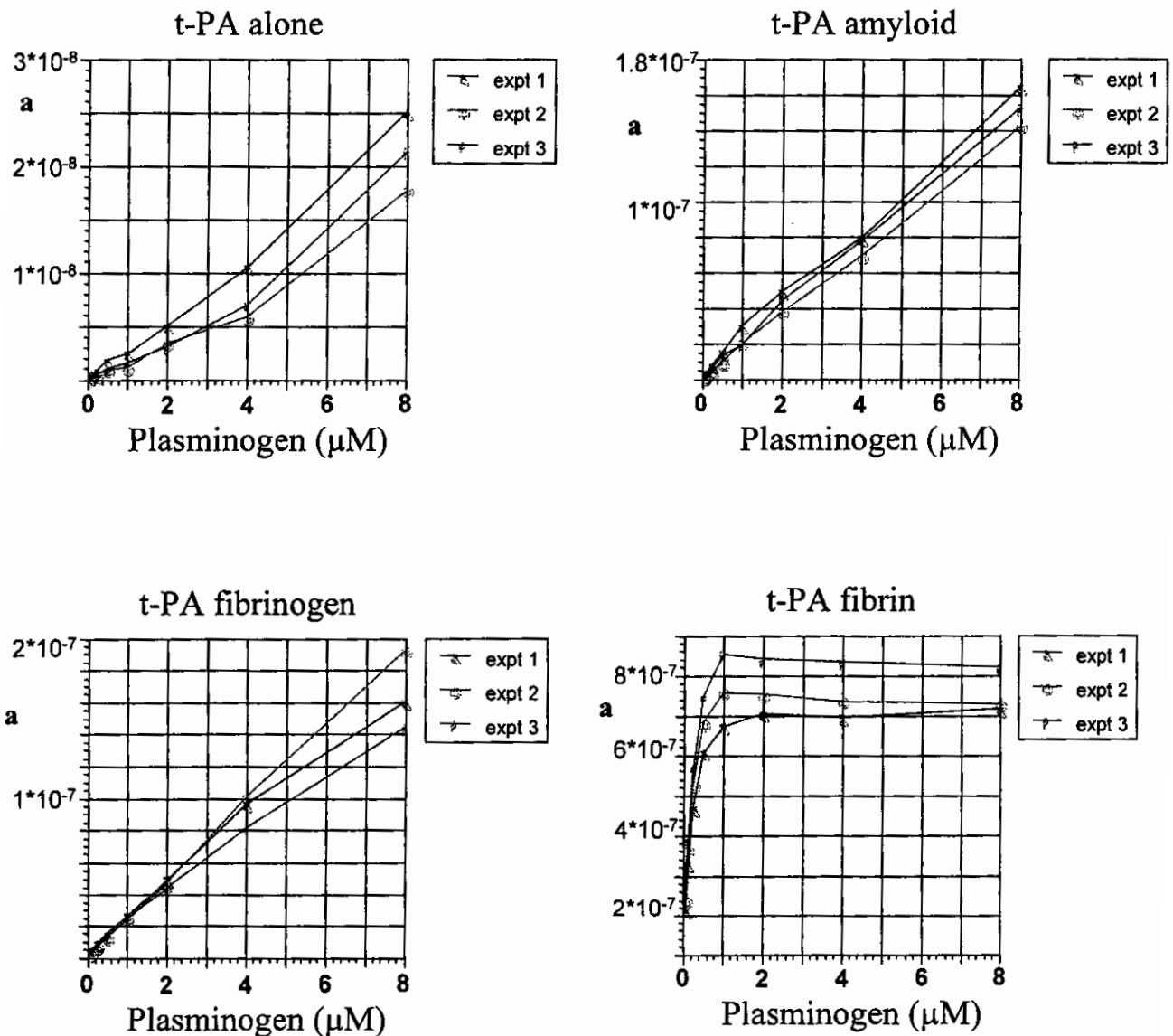


Figure 6a

Plots of a versus plasminogen concentration

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

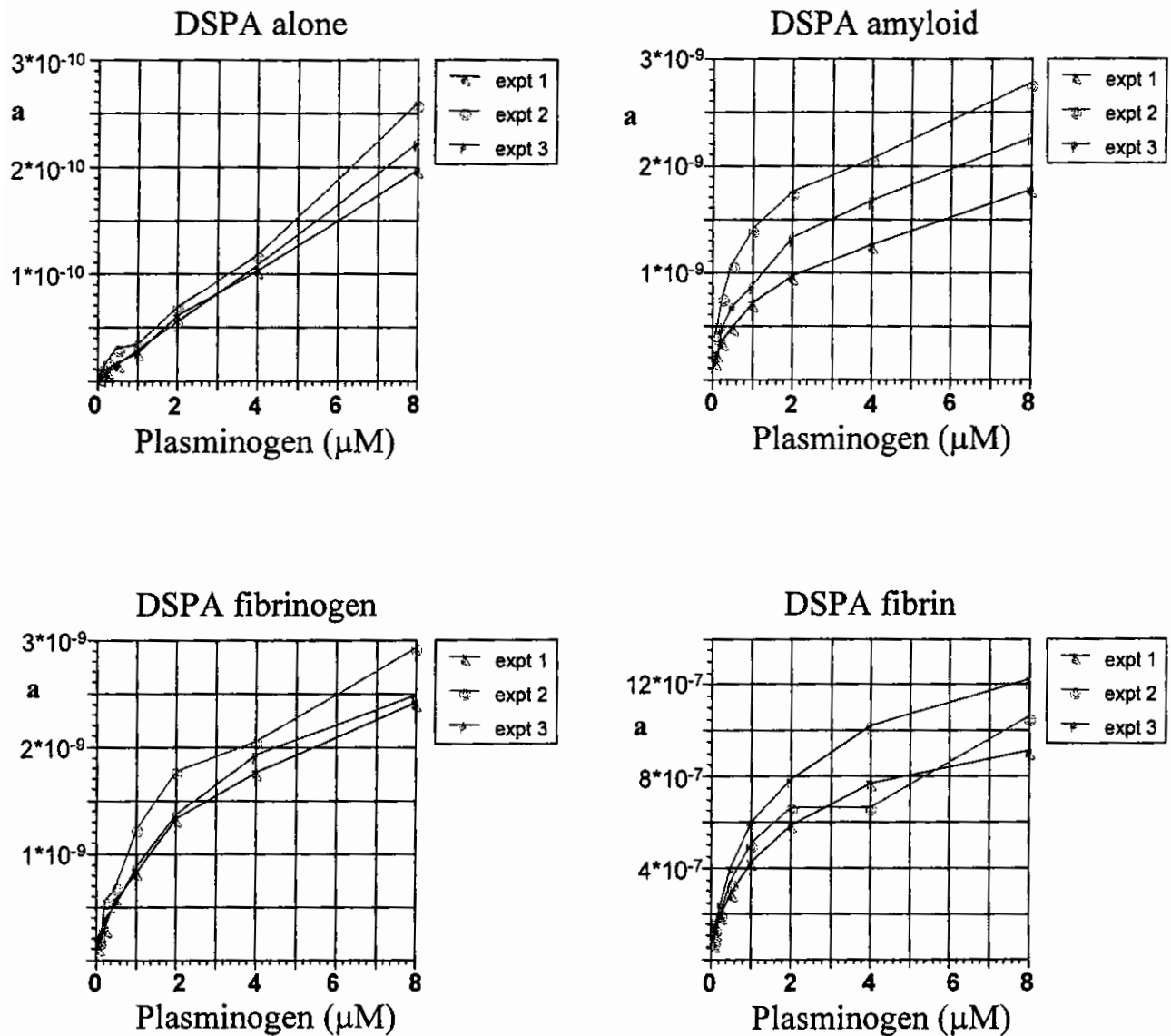


Figure 6b

Fig. 7

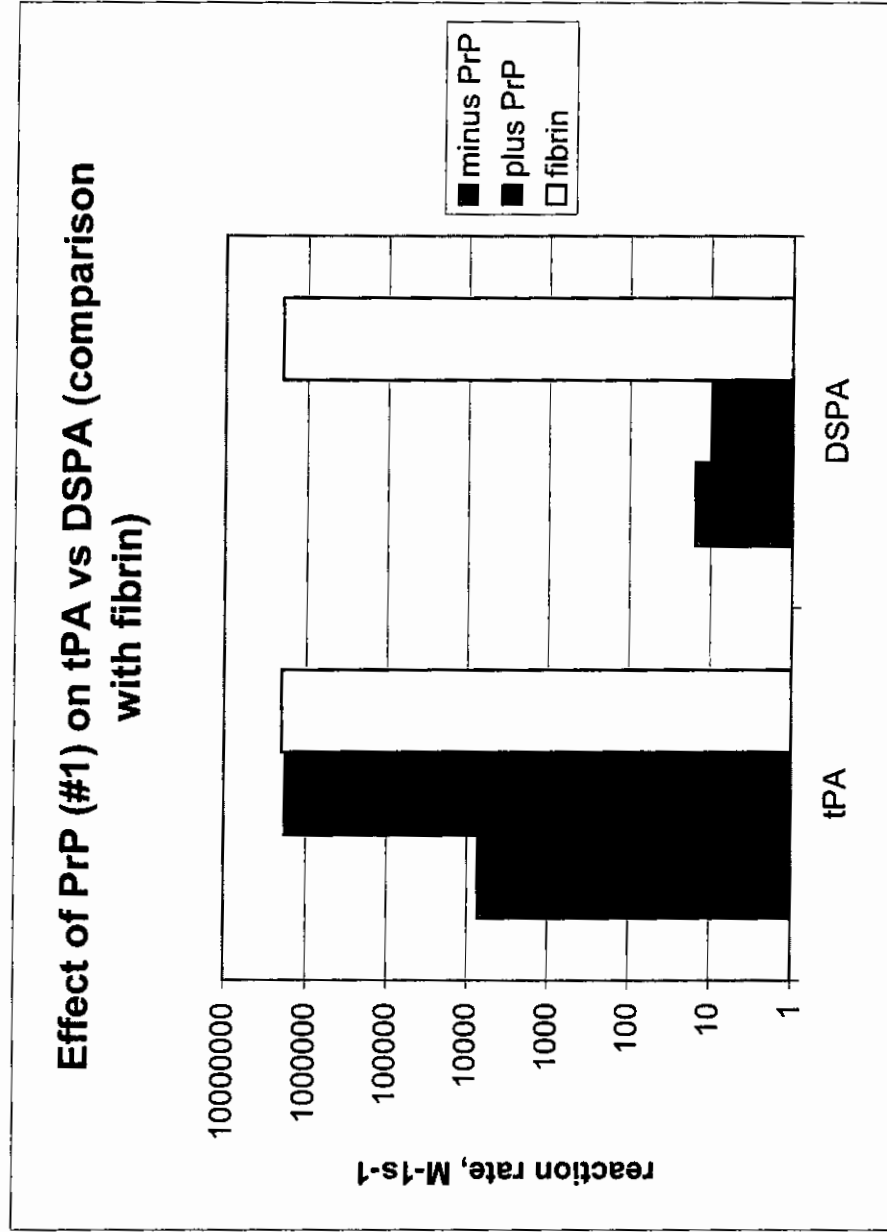


Fig. 8

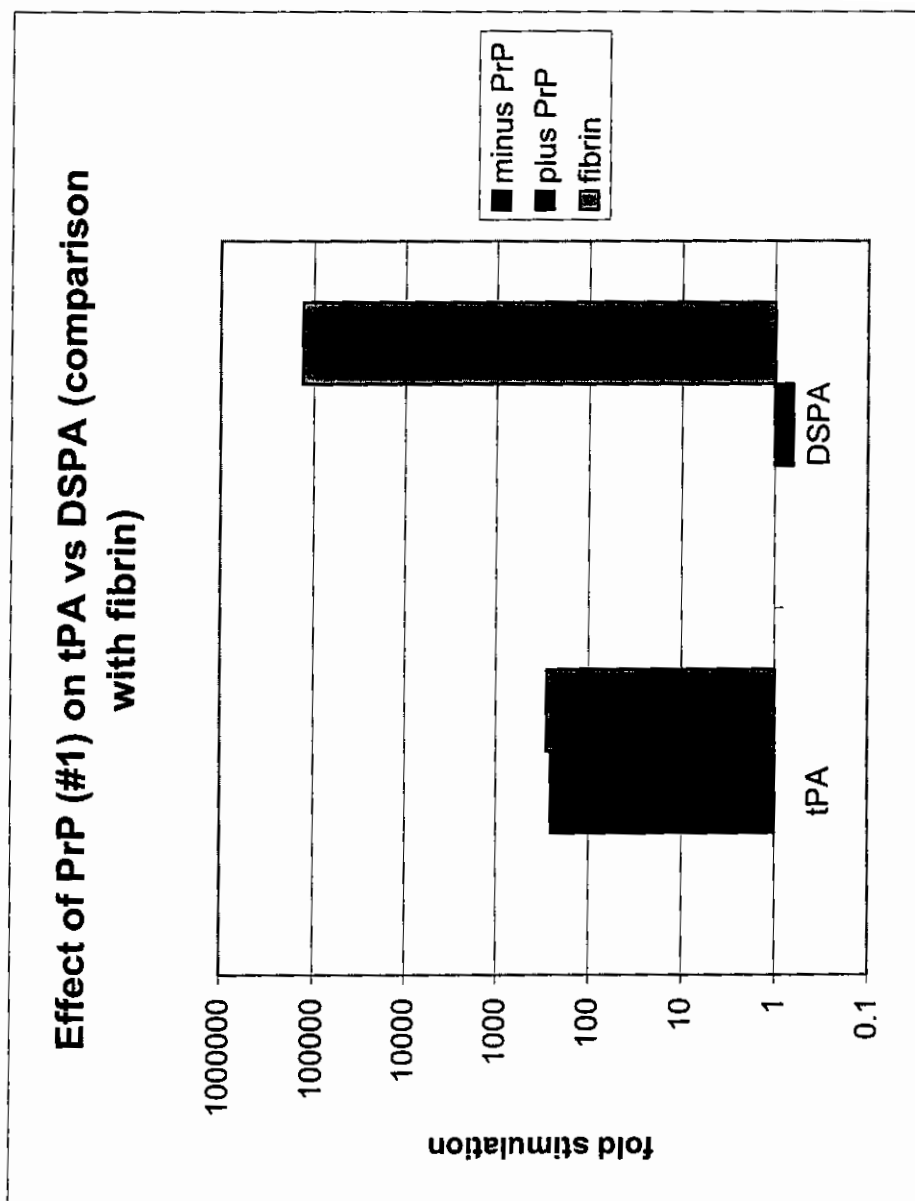


Fig. 9

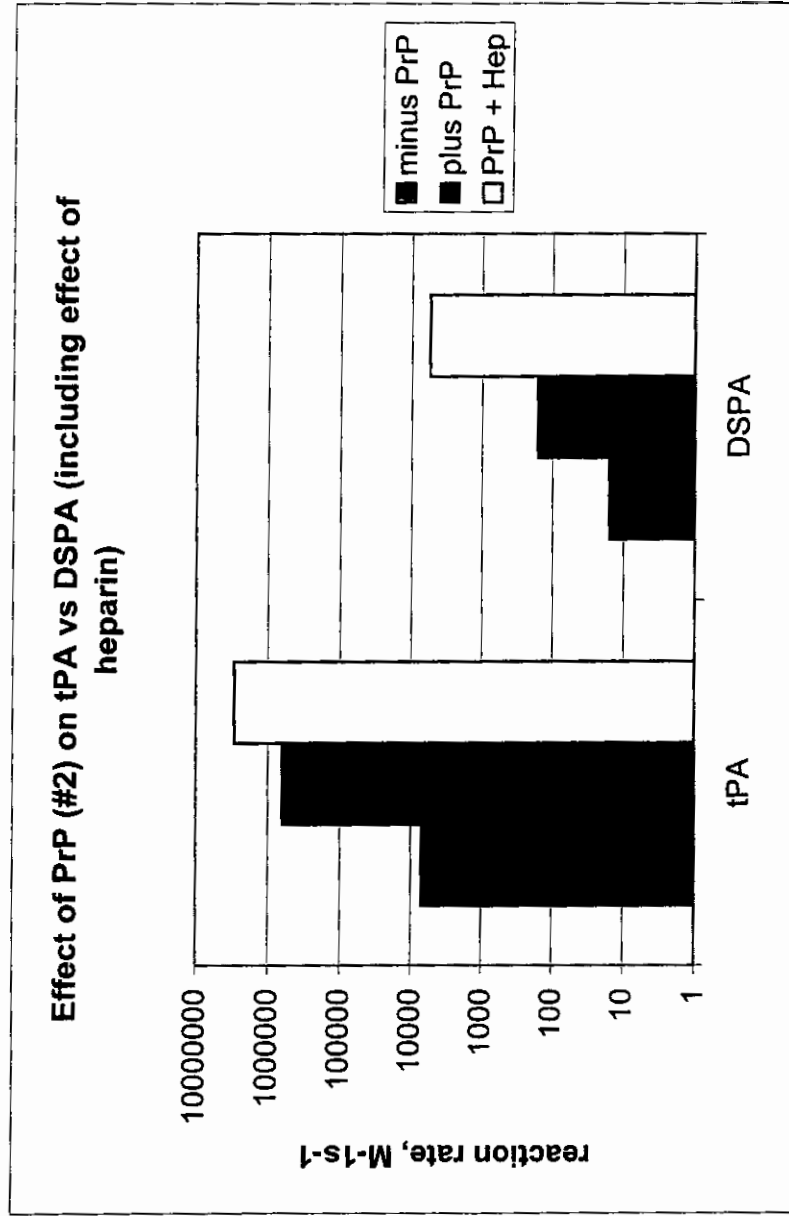


Fig. 10

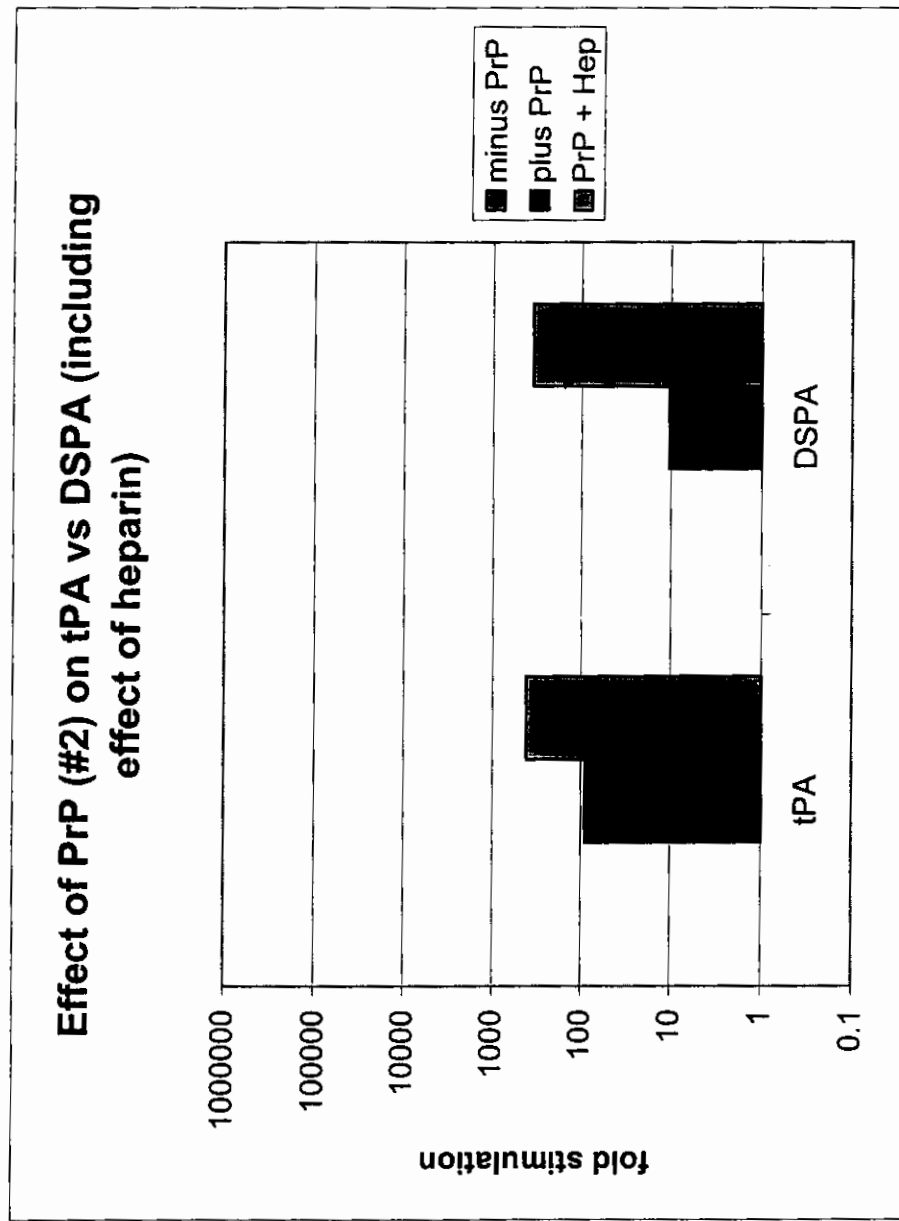


Fig. 11

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TSQHLLNRTVTDNMLCAGDTRSGGPQANLHDACQGDSSGGLVCLNDGRMTLVGIIISWGLGCGQKDVPGVYTKVTNYLD
WIRDNM RP

Fig. 12

tPA	M-DAMKRGLCCVLLLCGAVFVSPSQEIHFRRGARSYQVICRDEKTQMIYQQHQSWLRP	59
Fig. 3	M-DAMKRGLCCVLLLCGAVFVSPSQEIHFRRGARSYQVICRDEKTQMIYQQHQSWLRP	59
Fig. 11	M-DAMKRGLCCVLLLCGAVFVSPSQEIHFRRGARSYQVICRDEKTQMIYQQHQSWLRP	59
DSPA_al	MVNTMKTKLLCVLLLCGAVFSLPQETRYQLARGSRAYGVACKDEITQMTYRRQESWLRP	60
	: :*: * ***** * * : : * *: * * * *: * * * * *: :*:*****	
tPA	VLRNVRVEYCWNSGRAQCHSVPVKSCSEPRCFNGGTCQQALYFSDFVCQCPEGFAGKCC	119
Fig. 3	VLRNVRVEYCWNSGRAQCHSVPVKSCSEPRCFNGGTCQQALYFSDFVCQCPEGFAGKCC	119
Fig. 11	VLRNVRVEYCWNSGRAQCHSVPVKSCSEPRCFNGGTCQQALYFSDFVCQCPEGFAGKCC	119
DSPA_al	EVRSKRVEHCQCDRGQARCHTVPVNSCSEPRCFNGGTCWQAVYFSDFVCQCPAGYTGKRC	120
	: *: *: *: * : * *: *: *: *: *: *: *: *: *: *: *: *: *: *: *: *: *	
tPA	EIDTRATCYEDQGISYRGTWSTAESGAECTNWNSSALAQKPYSGRRPDAIRLGLGNHNYC	179
Fig. 3	EIDTRATCYEDQGISYRGTWSTAESGAECTNWNSSALAQKPYSGRRPDAIRLGLGNHNYC	179
Fig. 11	EIDTRATCYEDQGISYRGTWSTAESGAECTNWNSSALAQKPYSGRRPDAIRLGLGNHNYC	179
DSPA_al	EVDTRATCYEGQGVTYRGTWSTAESRVECINWNSSLLTRRTYNGRMPDAFNLGLGNHNYC	180
	*:*****. *: :*****. * * * * * *: : : . * * * * * *: : .*****	
tPA	RNPDRDSKPWCYVFKAGKYSSEFCSTPACSEGNSDCYFGNGSAYRGTHSLTESGASCLPW	239
Fig. 3	RNPDRDSKPWCYVFKAGKYSSEFCSTPACSEGNSD-----	214
Fig. 11	RNPDRDSKPWCYVFKAGKYSSEFCSTPACSK-----	210
DSPA_al	RNPNGAPKPWCYVIKAGKFTSESCVPVCSK-----	211
	: . **: *****: * * * * * . * * *	
tPA	NSMILIGKVYTAQNPSAQALGLGKHNYCRNPDGDAKPWCHVLKNRRLTWEYCDVPSCSTC	299
Fig. 3	-----STC	217
Fig. 11	-----ATC	213
DSPA_al	-----ATC	214
	: **	
tPA	GLRQYSQPQFRIKGGFLFADIASHPWQAAIFAKHRRSPGERFLCGGILISSCWILSAAHCF	359
Fig. 3	GLRQYSQPQFHSKGGFLFADIASHPWQAAIFAKHRRSPGERFLCGGILISSCWILSAAHCF	277
Fig. 11	GLRQYSQPQLHSTGGFLFADIASHPWQAAIFAKHRRSPGERFLCGGILISSCWILSAAHCF	273
DSPA_al	GLRKYKEPQLHSTGGFLFTDITSHPWQAAIFAQNRSSGERFLCGGILISSCWVLTAAHCF	274
	: . *: *: : . **: *: *****: *: *****: *: *****	
tPA	QERFPPHHLTIVILGRTYRVVPGEEEQKFEVEKYIVHKEFDDDTYDNDIALQLKSDSSRC	419
Fig. 3	QERFPPHHLTIVILGRTYRVVPGEEEQKFEVEKYIVHKEFDDDTYDNDIALQLKSDSSRC	337
Fig. 11	QERFPPHHLTIVILGRTYRVVPGEEEQKFEVEKYIVHKEFDDDTYDNDIALQLKSDSSRC	333
DSPA_al	QESYLPDQLKVVLGRTYRVKPGEEQTFKVKKYIVHKEFDDDTYNNDIALQLKSDSPQC	334
	* * : * . *: *: ***** * * * * * *: *: *****: *****: * *	
tPA	AQESSVVRTVCLPPADLQLPDWTECELSGYGKHEALSPFYSERLKEAHVRLYPSSRCTSQ	479
Fig. 3	AQESSVVRTVCLPPADLQLPDWTECELSGYGKHEALSPFYSERLKEAHVRLYPSSRCTSQ	397
Fig. 11	AQESSVVRTVCLPPADLQLPDWTECELSGYGKHEALSPFYSERLKEAHVRLYPSSRCTSQ	393
DSPA_al	AQESDSVRAICLPEANLQLPDWTECELSGYGKHKSSSPFYSEQLKEGHVRLYPSSRCAPK	394
	. *: : * * * * * *: **: : * * * * * *: * * . *****: : *	
tPA	HLLNRTVTDNMLCAGDTRSGGPQANLHDACQGDGGPLVCLNDGRMTLVGIIISWGLGCGQ	539
Fig. 3	HLLNRTVTDNMLCAGDTRSGGPQANLHDACQGDGGPLVCLNDGRMTLVGIIISWGLGCGQ	457
Fig. 11	HLLNRTVTDNMLCAGDTRSGGPQANLHDACQGDGGPLVCLNDGRMTLVGIIISWGLGCGQ	453
DSPA_al	FLFNKTVTNMLCAGDTRSGEIPNVHDACQGDGGPLVCMNDNHMTLLGIIISWGVGCGE	454
	. *: *: *: ***** . *: *****: * * . *: *****: * * :	
tPA	KDVPGVYTKVTNYLDWIRDNM RP	562
Fig. 3	KDVPGVYTKVTNYLDWIRDNM RP	480
Fig. 11	KDVPGVYTKVTNYLDWIRDNM RP	476
DSPA_al	KDVPGVYTKVTNYLGWIRDNMHL	477

Fig. 13

MDAMKRGLCCVLLLCGAVFSLPSQETHRQLARGSRAYQVACRDEITQMIYQQHQSWLRPVLRSKRVEHCQCNRGQARC
HSVPVKSCSEPRCFNGGTCWQALYFSDFVCQCPEGYTGKCCEIDTRATCYEGQGISYRGTWSTAESRVECTNWNSSL
TRRTYNGRMPDAFNLGLGNHNYCRNPNGAPKPWCYVIKAGKFSSSESCSVPACSKATCGLRKYKEPQLHSTGGLFTDIA
SHPWQAAIFAQHRRSSGERFLCGGILISSCWILSAAHCFQESYLPDQLKVVLGRTYRVVPGEETFKVKKYIVHKEF
DDDTYDNDIALQLKSDSPQCAQESDSVRAICLPEANLQLPDWTECELSGYGKHESSEPFYSEQLKEGHVRLYPSSRC
APKFLFNRTVTNNMLCAGDTRSGEIYPNVHDACQGDGGPLVCMNDNHMTLVGIIISWGVGCGQKDVPGVYTKVTNYLG
WIRDNMHL

FIG 14

S-2288 Activity of vamptPA-SPXL-Column Fractions

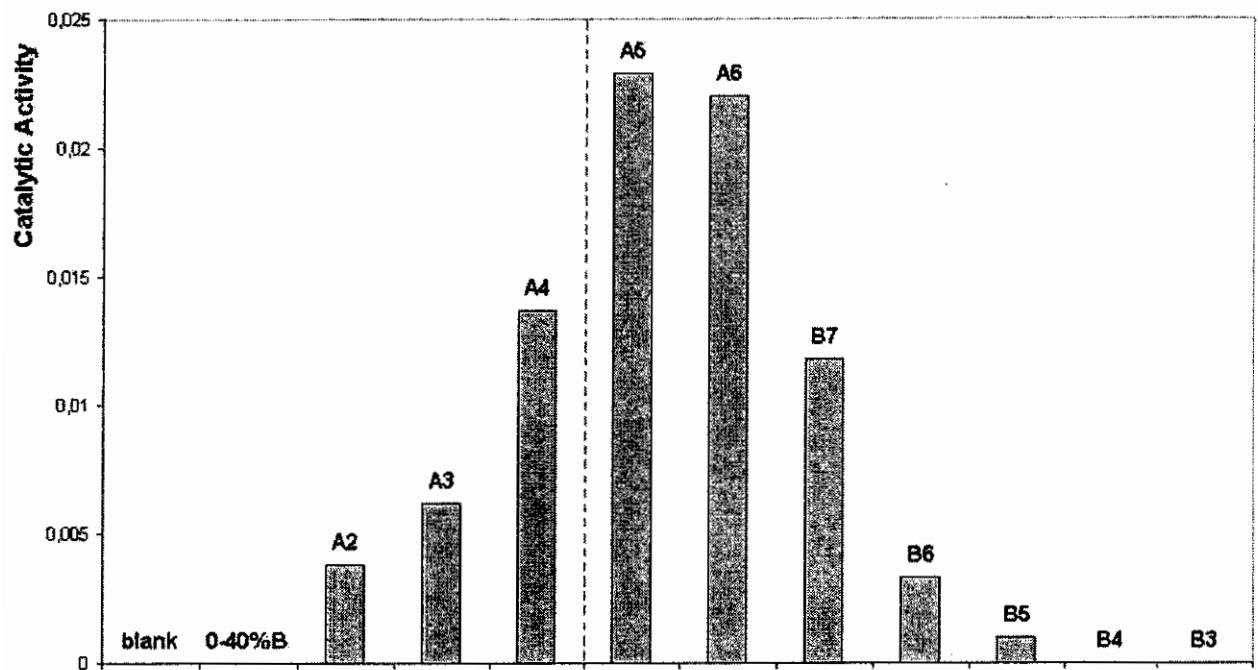
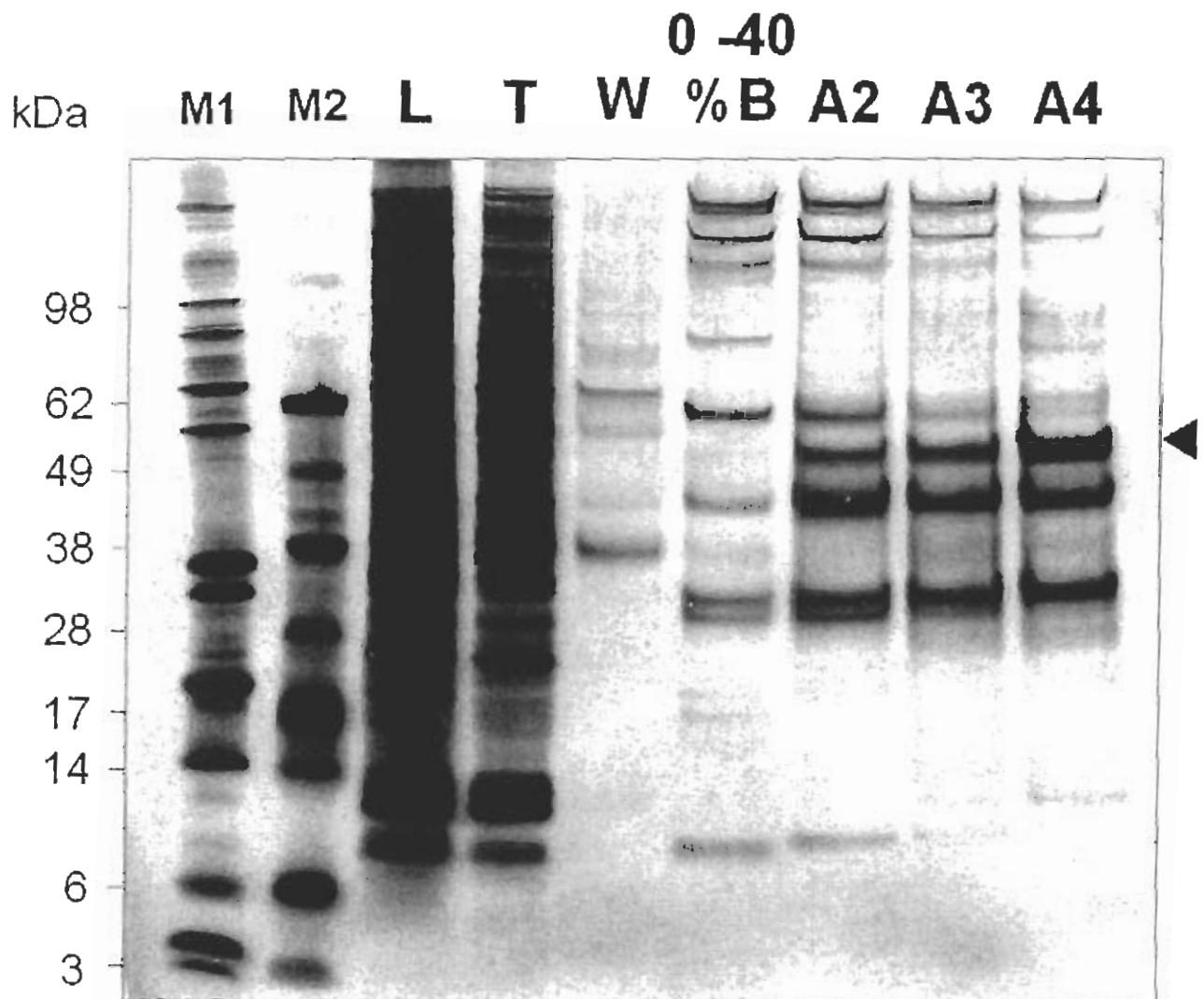


FIG 15 a

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



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

kDa M1 M2 **A5** **A6** **B7** **B6** **B5** **B4**

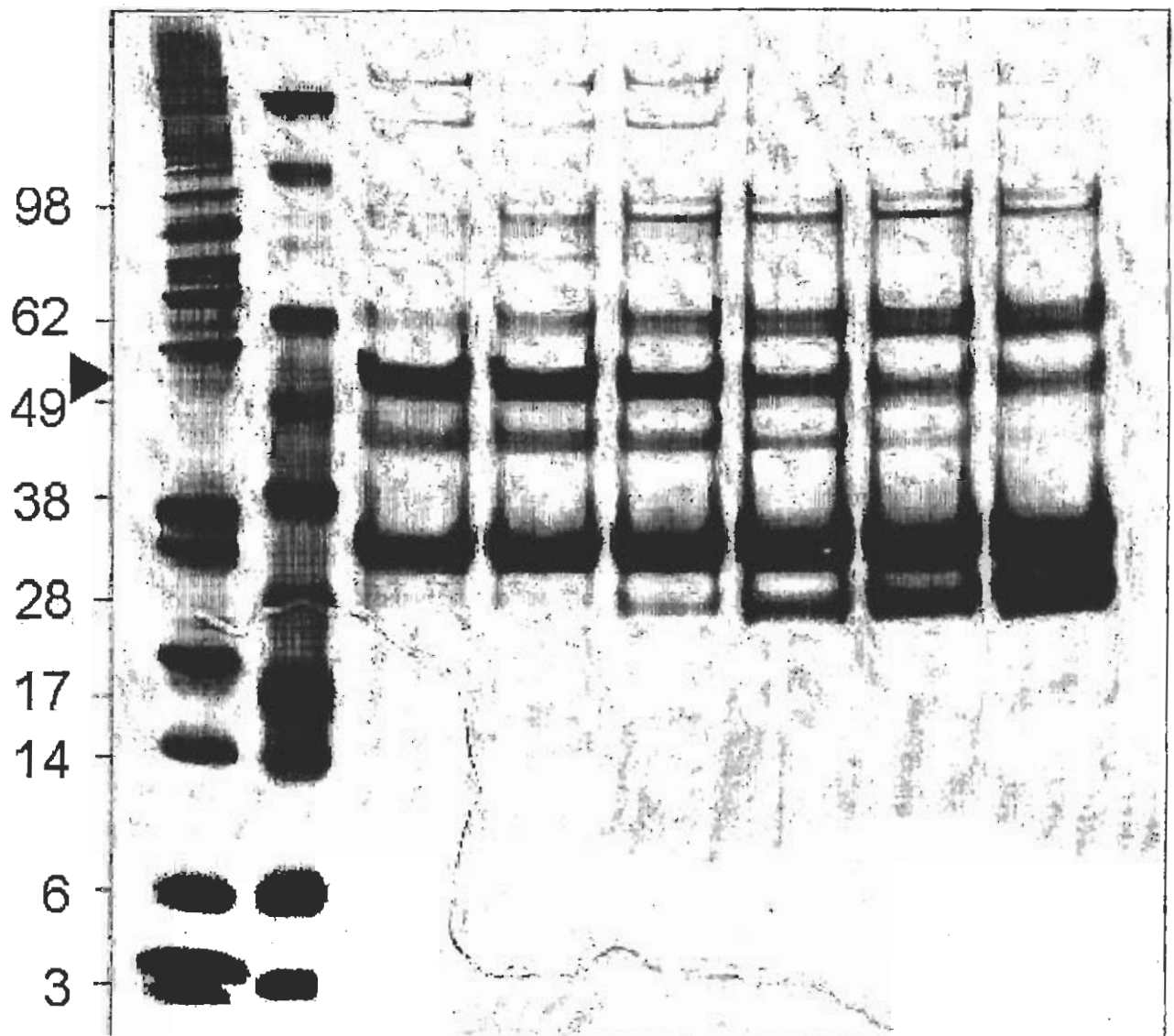


FIG 16 a

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Western Blot of SPXL-column fractions

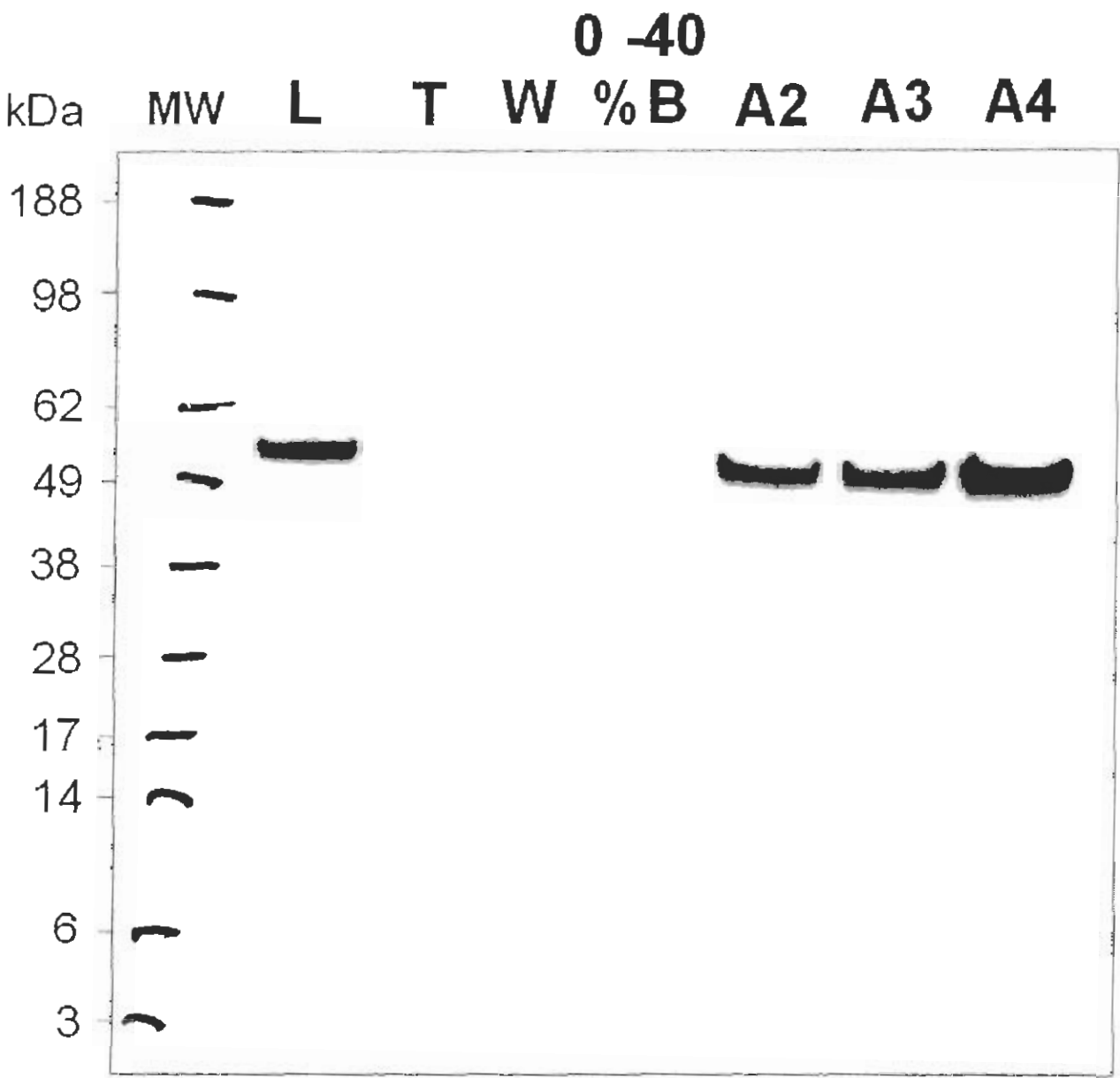


FIG 16 b

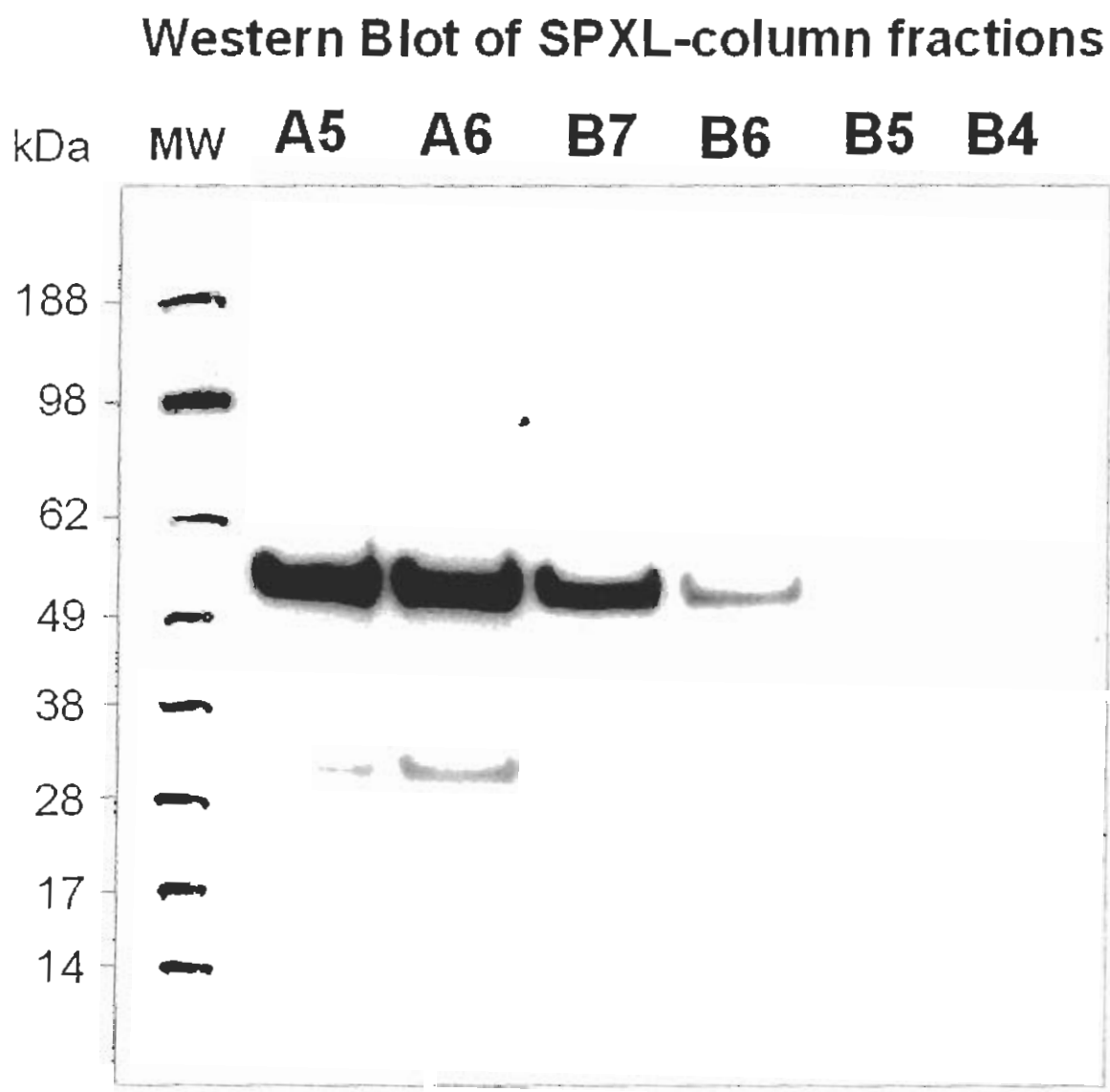


FIG 17 a

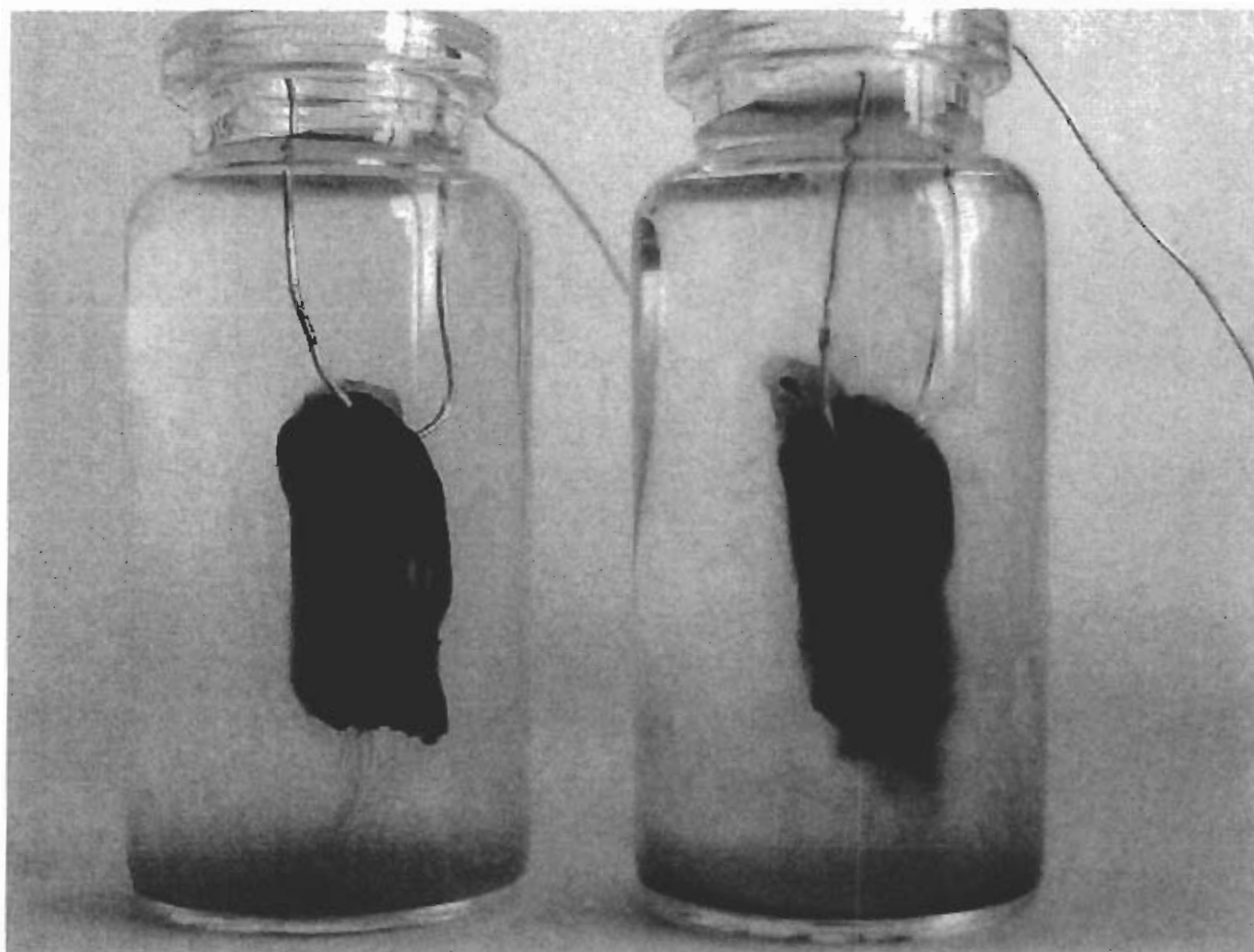


FIG 17 b

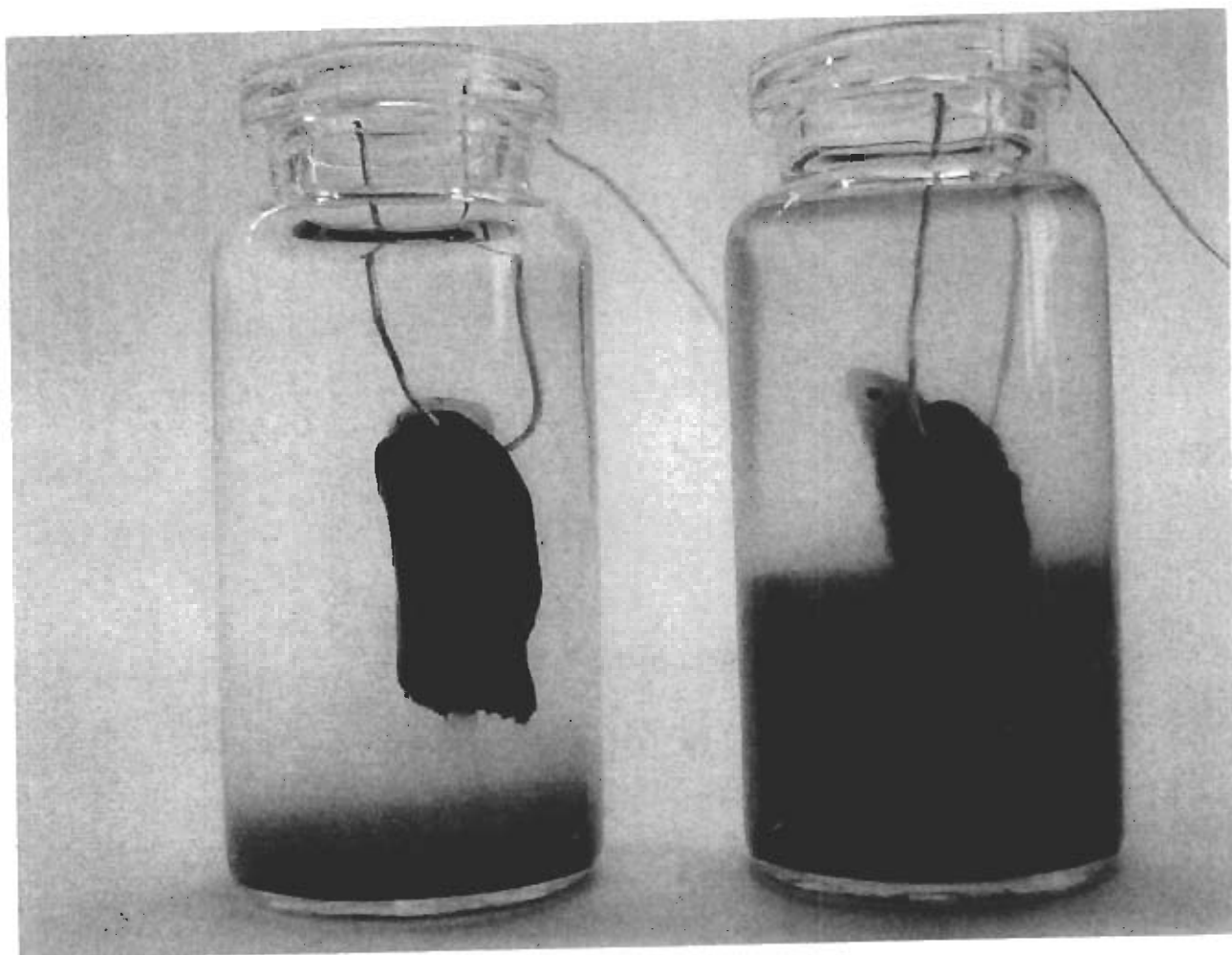


FIG 17 c

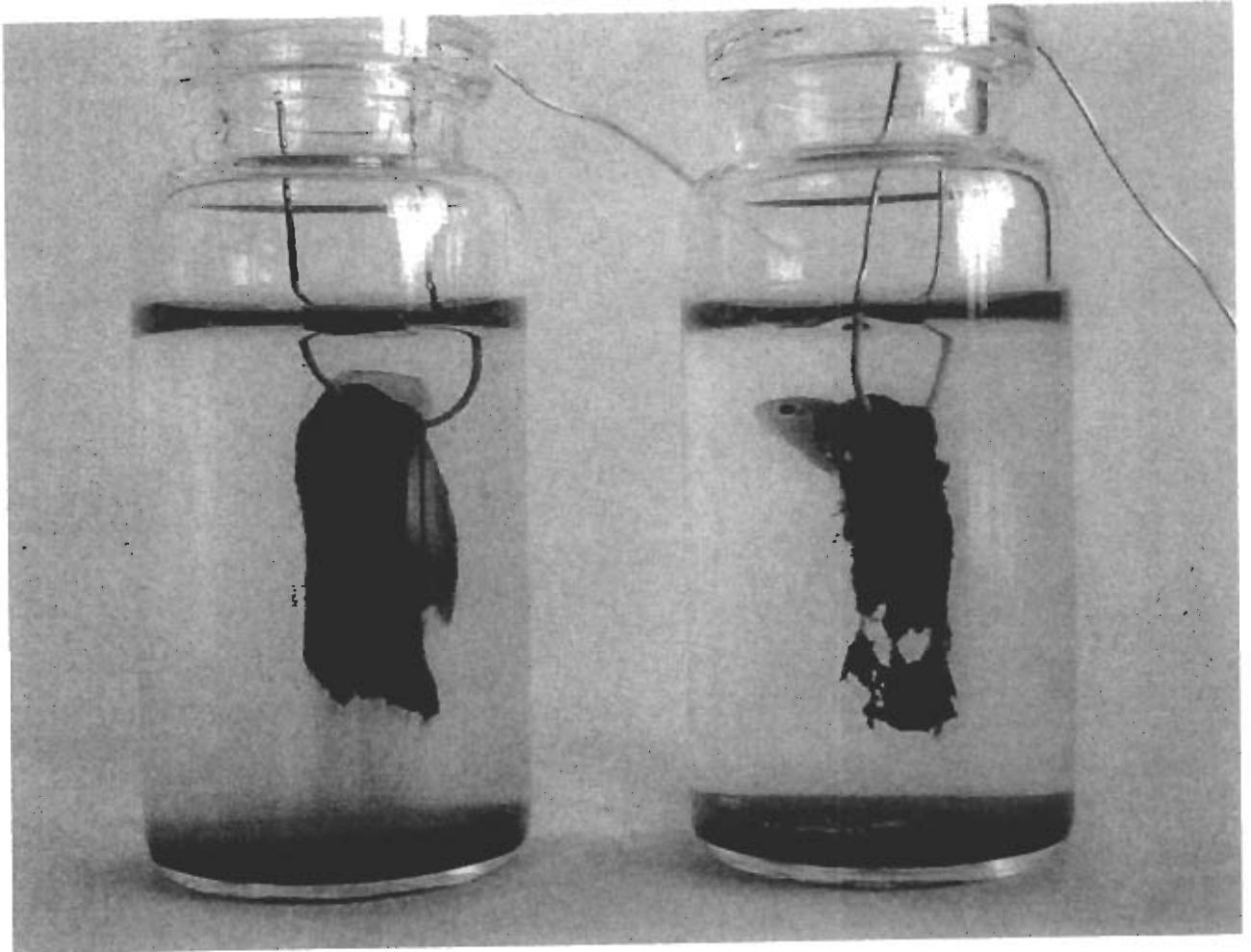


FIG 17 d

