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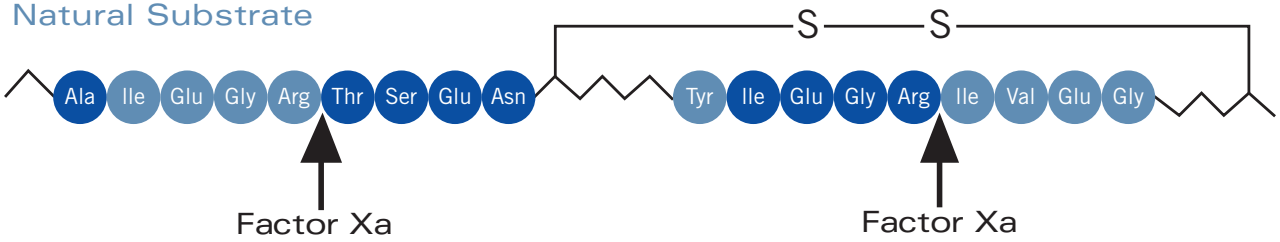
CHROMOGENIC SUBSTRATES

DIAPHARMA CHROMOGENIC SUBSTRATES

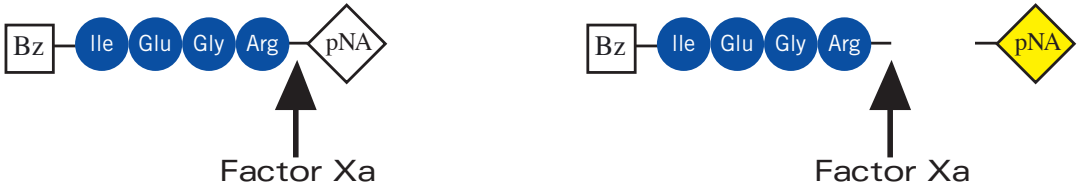
Substrate	Analyte	Method	Units	Cat #
Chromogenix S-2222*	Factor Xa	Chromogenic	25 mg	S820316
Chromogenix S-2765*	Factor Xa	Chromogenic	25 mg	S822254
DiaPharma CS - FXa†	Factor Xa	Chromogenic	5 mg	DPG765-5
Chromogenix S-2238*	Thrombin	Chromogenic	25 mg	S820324
DiaPharma CS - TH†	Thrombin	Chromogenic	5 mg	DPG238-5
DiaPharma CS - TG†	Thrombin-Generation Chromogenic Substrate	Chromogenic	10 µmol	DPG239-10
Chromogenix S-2302*	Plasma Kallikrein, Factor XIIa	Chromogenic	25 mg	S820340
Chromogenix S-2366*	Activated Protein C, Factor XIa	Chromogenic	25 mg	S821090
Chromogenix S-2288*	t-PA and a broad spectrum of other Serine Proteases	Chromogenic	25 mg	S820852
Chromogenix S-2251*	Plasmin, Streptokinase-activated Plasminogen	Chromogenic	25 mg	S820332
Chromogenix S-2403*	Plasmin, Streptokinase-activated Plasminogen	Chromogenic	25 mg	S821413
DiaPharma CS - UK†	Urokinase	Chromogenic	25 mg	DPG444-25
DiaPharma CS - CHY†	Chymotrypsin	Chromogenic	25 mg	DPG586-25
DiaPharma CS - KAL†	Kallikrein, FXIa	Chromogenic	25 mg	DPG266-25

Prothrombin Cleaved By Factor Xa

Natural Substrate



SYNTHETIC SUBSTRATE (S-2222)



Prothrombin, the natural substrate of Factor Xa, is cleaved by Factor Xa at two positions, each proceeded by the same four amino acid sequence. Factor Xa activity can be determined by the chromogenic substrate S-2222*, which is composed of the same amino acids coupled to a chromophore.

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PRODUCTS

S-2222™*

Catalog #: S820316

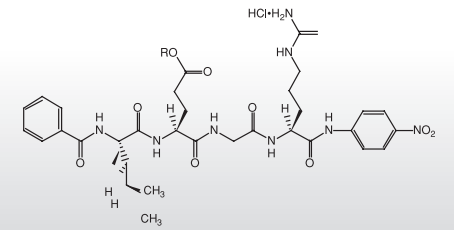
Analytes:
+ Factor Xa

Available in sizes:
+ 25 mg

Potential Applications:
+ Human and Bovine Factor Xa
+ Human and Bovine Factor IX
+ Human and Bovine Factor X
+ Factor Xa Inhibitors

Formula:
Bz-Ile-Glu(γ-OR)-Gly-Arg-pNA•HCl

Molecular Weight:
741.3



S-2765™*

Catalog #: S821413

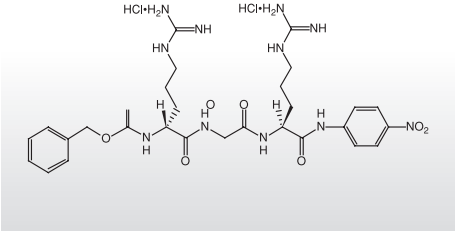
Analytes:
+ Factor Xa

Available in sizes:
+ 25 mg

Potential Applications:
+ Human and Bovine Factor Xa
+ Human and Bovine Factor X
+ Human Factor IX
+ Factor Xa Inhibitors

Formula:
Z-D-Arg-Gly-Arg-pNA•2HCl

Molecular Weight:
714.6



DiaPharma CS - FXa†

Catalog #: DPG765-5MG

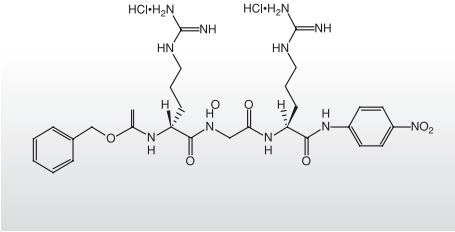
Analytes:
+ Factor Xa

Available in sizes:
+ 5 mg
+ Bulk (special order only)

Potential Applications:
+ Human and Bovine Factor Xa
+ Human and Bovine Factor X
+ Human Factor IX
+ Factor Xa Inhibitors

Formula:
Z-D-Arg-Gly-Arg-pNA•2HCl

Molecular Weight:
714.6



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S-2238™*

Catalog #: S820324

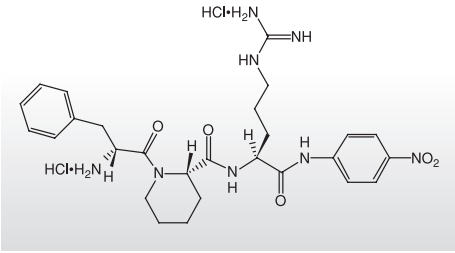
Analytes:
+ Thrombin

Available in sizes:
+ 25 mg

Potential Applications:
+ Human and Bovine Thrombin
+ Human and Bovine Antithrombin
+ Human and Bovine Prothrombin
+ Factor IIa Inhibitors

Formula:
H-D-Phe-Pip-Arg-pNA•2HCl

Molecular Weight:
625.6



DiaPharma CS - TH†

Catalog #: DPG238-5

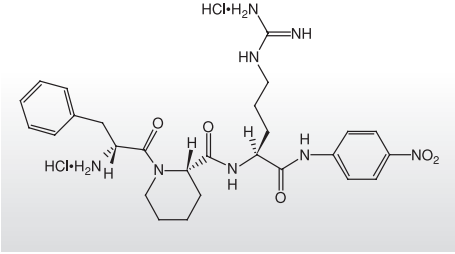
Analytes:
+ Thrombin

Available in sizes:
+ 5 mg
+ Bulk (special order only)

Potential Applications:
+ Human and Bovine Antithrombin
+ Human and Bovine Prothrombin
+ Human and Bovine Thrombin
+ Factor IIa Inhibitors

Formula:
H-D-Phe-Pip-Arg-pNA•2HCl

Molecular Weight:
625.6



DiaPharma CS - TG†

Catalog #: DPG239-10

Analytes:
+ Thrombin (slow rate for continuous registration of thrombin formation in plasma)

Available in sizes:
+ 10 µmol
+ Bulk (special order only)

Potential Applications:
+ Human Thrombin
+ Thrombin Generation Assays

Formula:
H-B-Ala-Gly-Arg-pNA•2AcOH

Molecular Weight:
542.6

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PRODUCTS

S-2302™*

Catalog #: S820340

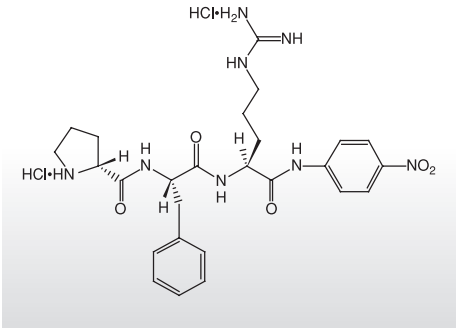
Analytes:
+ Plasma Kallikrein
+ Factor XIIa

Available in sizes:
+ 25 mg

Potential Applications:
+ Human Kallikrein
+ Human Prekallikrein
+ Prekallikrein Activator
+ Human Factor XIa

Formula:
H-D-Pro-Phe-Arg-pNA•2HCl

Molecular Weight:
611.6



S-2366™*

Catalog #: S821090

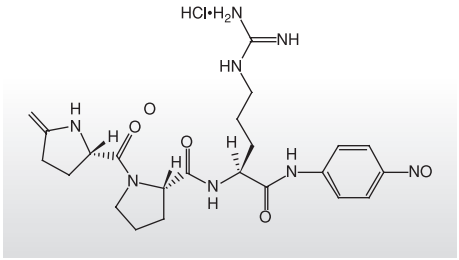
Analytes:
+ Activated Protein C
+ Factor XIa

Available in sizes:
+ 25 mg

Potential Applications:
+ Human and Bovine APC
+ Human and Bovine Protein C
+ Human and Bovine XIa

Formula:
pyroGlu-Pro-Arg-pNA•HCl

Molecular Weight:
539.0



S-2288™*

Catalog #: S820852

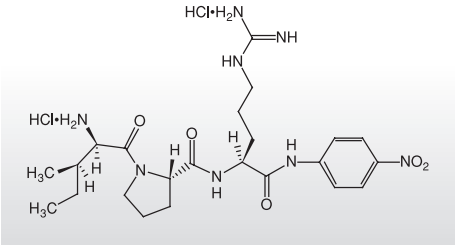
Analytes:
+ Tissue Plasminogen Activator
+ Other Serine Proteases

Available in sizes:
+ 25 mg

Potential Applications:
+ Human tPA
+ Serine Proteases (purified systems)

Formula:
H-D-Ile-Pro-Arg-pNA•2HCl

Molecular Weight:
577.6



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S-2251™*

Catalog #: S820332

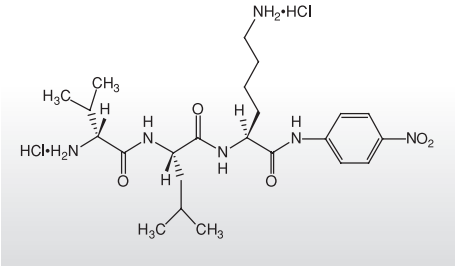
Analytes:
+ Plasmin
+ Streptokinase-activated Plasminogen

Available in sizes:
+ 25 mg

Potential Applications:
+ Human and Bovine Plasmin
+ Human and Bovine Plasminogen
+ Plasminogen/Streptokinase Complex

Formula:
H-D-Val-Leu-Lys-pNA•2HCl

Molecular Weight:
551.6



S-2403™*

Catalog #: S822254

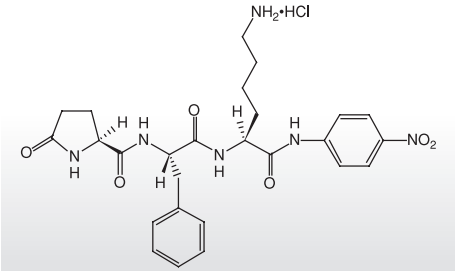
Analytes:
+ Plasmin
+ Plasminogen-SK

Available in sizes:
+ 25 mg

Potential Applications:
+ Human and Bovine Plasmin
+ Human and Bovine Plasminogen
+ Plasminogen/Streptokinase Complex

Formula:
pyroGlu-Phe-Lys-pNA•HCl

Molecular Weight:
561.0



DiaPharma CS - UK†

Catalog #: DPG444-25

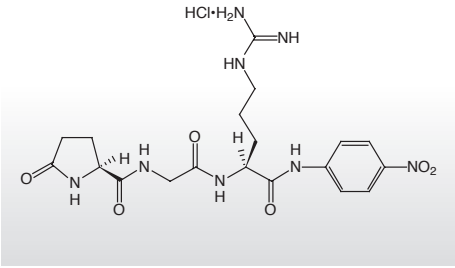
Analytes:
+ Urokinase

Available in sizes:
+ 25 mg
+ Bulk (special order only)

Potential Applications:
+ Human Urokinase

Formula:
pyroGlu-Gly-Arg-pNA•HCl

Molecular Weight:
498.9



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PRODUCTS

DiaPharma CS - CHY†

Catalog #: DPG586-25

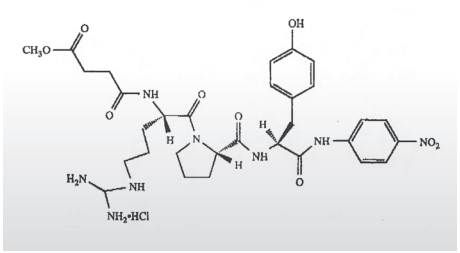
Analytes:
+ Chymotrypsin

Available in sizes:
+ 25 mg
+ Bulk (special order only)

Potential Applications:
+ Chymotrypsin

Formula:
MeO-Suc-Arg-Pro-Tyr-pNA•HCl

Molecular Weight:
668.29 (without HCl)



DiaPharma CS - KAL†

Catalog #: DPG266-25

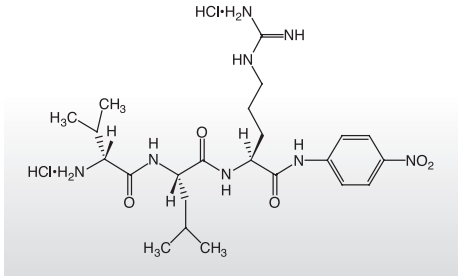
Analytes:
+ Glandular Kallikreins
+ Factor XIa

Available in sizes:
+ 25 mg
+ Bulk (special order only)

Potential Applications:
+ Kallikreins
+ Factor XIa

Formula:
H-D-Val-Leu-Arg-pNA-2HCl

Molecular Weight:
579.6



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KINETIC DATA (NATURAL VS. SYNTHETIC)

Serine Protease	Natural Substrate & Cleavage Sequence P9.....P4 - P3 - P2 - P1 - P1' - P2'	K_m (mM)	k_{cat} (s^{-1})	Synthetic Substrate	K_m (mM)	k_{cat} (s^{-1})
Thrombin (Bovine)	Fibrinogen Phe.....Gly-Gly-Val-Arg-Gly-Pro	0.012	71	S-2238™* D-Phe-Pip-Arg-pNA	0.010	200
Factor Xa (Bovine)	Prothrombin .....Ile-Glu-Gly-Arg-Thr-Ser	$0.04 \bullet 10^{-3*}$	75	S-2222™* Bz-Ile-Glu(g-OR)-Gly-Arg-pNA S-2765™* Z-D-Arg-Gly-Arg-pNA	0.40 0.11	100 195
Plasma Kallikrein (Human)	HMW Kininogen Arg.....Ser-Pro-Phe-Arg-Ser-Val	$3.7 \bullet 10^{-3}$	3.2	S-2302™* D-Pro-Phe-Arg-pNA	0.22	150
Plasmin (Human)	Fibrin Tyr.....Glu-Thr-Lys-Val-Asp	$6.5 \bullet 10^{-3}$	7.1	S-2251™* D-Val-Leu-Lys-pNA S-2403™* pyroGlu-Phe-Lys-pNA	0.40 0.35	20 60
Activated Protein C (Human)	Factor Va .....Leu-Asp-Arg-Arg-Gly-Ile	$20 \bullet 10^{-6}$	0.4	S-2366™* pyroGlu-Pro-Arg-pNA	0.20	190

Kinetic data for some natural and chromogenic substrates of serine proteases involved in blood coagulation and fibrinolysis.
In the presence of phospholipid and FVa.

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WHAT IS A CHROMOGENIC SUBSTRATE?

Enzymes are proteins that catalyze most of the chemical reactions that take place in the body. They make it possible for chemical reactions to occur at neutral pH and body temperature. The chemical compound upon which the enzyme exerts it's catalytic activity is called a substrate. Proteolytic enzymes act on their natural substrates, proteins and peptides, by hydrolyzing one or more peptide bond(s). This process is usually highly specific in the sense that only peptide bonds adjacent to certain amino acids are cleaved. Chromogenic substrates are peptides that react with proteolytic enzymes with the formation of color. They are made synthetically and are designed to possess a selectivity similar to that of the natural substrate for the enzyme. Attached to the peptide part of the chromogenic substrate is a chemical group when released after the enzyme cleavage gives a color change. **The color change can be followed spectrophotometrically and is proportional to the proteolytic activity.**

The chromogenic substrate technology was developed in the early 1970s, and has since then become a routine tool in basic research.

ENZYME SPECIFICITY AND SUBSTRATE SELECTIVITY

SPECIFICITY is a property of the enzyme and describes how restrictive the enzyme is in its choice of substrate; a completely specific enzyme would have only one substrate.

The specificity of the serine proteases is usually not very high since they have similar active sites and act through the same proteolytic mechanism. Consequently, a single serine protease may act on various substrates although at different rates.

How the substrate fits the active site of the enzyme is of crucial importance to the outcome of the enzyme-substrate reaction. The bond to be cleaved must have a specific orientation relative to the amino acid side-chains of the catalytic triad.

The most important factor governing the fit of a substrate for an enzyme is the amino acid sequence around the bond to be cleaved. Trypsin cleaves amides and esters of the basic amino acids arginine and lysine. Thrombin has a similar preference, but is more specific for arginine than for lysine.

SELECTIVITY is a property of the substrate and indicates the degree to which the substrate is bound to and cleaved by different enzymes. The best measure for selectivity is given by the ratio kcat/Km.

Synthetic substrates are considerably smaller than the natural substrates and can usually be cleaved by more than one enzyme, i.e., synthetic substrates are not completely selective. The explanation for this is that large substrates such as fibrinogen not only interact with the active site but also with exterior domains of the enzyme. Such interactions allow substrates to discriminate between different serine proteases, and fibrinogen thus becomes highly selective for thrombin.

CHROMOGENIC SUBSTRATES IN PRACTICE

Measurements made using chromogenic substrates reflect enzyme activity. Often it is more important to have knowledge about the activity of an enzyme rather than the amount of protein present as found in an immunological assay.

Synthetic substrates are very sensitive, i.e., they can detect very low enzyme activities. They are in fact often more sensitive than a corresponding natural substrate.

This ability of chromogenic substrates to detect low enzyme concentrations makes them useful in, for example, the search for the presence of certain enzyme activities either in research or in quality control procedures. Sometimes there is a lack in correspondence between a natural and a chromogenic substrate in their responses to a certain enzyme preparation. For example, thrombin that has been partly degraded through autohydrolysis (ß-thrombin) reacts just as well with its chromogenic substrate as does the native form of thrombin (α-thrombin), while only native thrombin reacts with the natural substrate fibrinogen.

SETTING UP CHROMOGENIC SUBSTRATE ASSAYS

A chromogenic substrate is less selective, i.e., it has less discrimination in its reactivity toward related enzymes compared to the natural substrate. However, this lack of absolute selectivity can be compensated for when setting up chromogenic substrate assays. This is done by the proper selection of type of buffer, pH, relative concentrations of sample and reagents, addition of inhibitors, and/or choice of activator or incubation times. When presented with the opportunity of using one or more chromogenic substrates in a particular experimental setting for which there is no existing method, there are a few considerations that are worthwhile to make.

Substrate: If the specificity of the enzymatic activity to be measured is known, then comprehensive overviews such as the Tables 1 and 3 on pages 12-15 will serve as a guide in selecting a proper substrate. The local distributor of Chromogenix products may also be contacted for advice on the choice of substrate(s).

SCREENING PROCEDURE If the specificity of the enzyme is unknown, a screening procedure can be applied. When different substrates are available, such screening of the enzyme specificity can be carried out by comparing the rate of hydrolysis or pNA-generation obtained with the different substrates. Unless certain experience is available to the investigators, it is usually advisable to discuss the plan and/or the result with Chromogenix. Advice on the next step can thus be given concerning either continued screening or the selection of a particular substrate that is suitable in the planned investigation.

Contaminating enzymes: If the sample to be tested with a chromogenic substrate contains more than one enzyme that may react with the same substrate, there are a number of measures that can be taken in order to eliminate the interfering/contaminating activity. A natural or synthetic inhibitor can be introduced, the sample can be further diluted or conditions can be found (different pH and/or buffer) where the relative activities of the present enzymes are optimized.

Such considerations can be based on the information below concerning temperature, pH, buffer and ionic strength.

Temperature: The rate by which the chromogenic substrate is cleaved is highly dependent on the temperature. It is therefore important to know at what temperature(s) a particular method is applicable – it may be at room (ambient) temperature, 25, 30, or 37 °C (body temp).

An increase in temperature of 1 °C causes an increase in the reaction velocity of 2.5-7.5%. The temperature thus must be kept constant during the measurement, and if results from different experiments are to be compared, they must be performed at the same temperature. It is advisable to run the reactions in temperature controlled cuvettes and to use preheated stock solutions.

pH: Both reaction rate and substrate turnover are dependent on the pH. This means that kinetic calculations can only be made using results obtained at the same pH.

Usually, the enzyme activity is measured at the pH optimum for the proteolytic activity of the enzyme. However, when several proteases are present in the same solution, as, e.g., when the sample is from plasma, it is not always advantageous to search for the pH that gives the maximum reactivity of the enzyme under investigation. Instead it is better to choose a pH where other serine proteases that may compete for the substrate have relatively lower levels of activity.

Buffers: The buffer type and the concentration of buffer substances must be well defined.

Usually Tris-HCl is used since the pKa of Tris buffer is 8.1 (25 °C), which makes it suitable for measurements at pH values between 7.3-9.3, where most of the serine proteases show maximal activities. Furthermore, this buffer is stable – it can even be autoclaved. Tris-imidazole has also been used, but is not to be recommended as imidazole is known to slightly inhibit certain proteases such as trypsin and plasmin.

Ionic strength and other additives: The appropriate ionic strength is usually obtained by adjusting the concentration of NaCl. Further substances that it may be necessary to add are CaCl2 (when Ca-dependent enzymes are studied), Sodium azide (or other bactericidal agents) to prevent bacterial growth and PEG, or Tween 80 to prevent adsorption of the enzymes to the reaction vessel walls.

Substrate handling: The substrate solution is usually prepared by adding sterile water to the dry powder. Substrates with low solubility in water can be dissolved in DMSO (dimethyl sulfoxide) and then diluted in water. The final DMSO concentration should preferably not exceed 10% in the reaction mixture.

STABILITY OF SUBSTRATE SOLUTIONS

Substrates dissolved in sterile water are stable for more than six months in the refrigerator (2-8 °C) and for several weeks at room temperature (25 °C). The stability is considerably reduced in alkaline buffers. Furthermore, contamination by microorganisms and exposure to light for longer periods should be avoided. The substrate concentration should be chosen so that linear kinetics is obtained. A substrate concentration of twice the Km (2 x Km) is usually appropriate.

TABLE 1

Enzyme	Substrate	Thrombin (B)	FXa (B)	FXIa (H)	APC (H)	Plasmin (H)	Single Chain t-PA (H)	Plasma Kallikrein (H)	C1s (H)	Buffer 50 mM Tris HCL pH	Buffer 50 mM Tris HCL NaCl (mM)	Substrate conc. 2xKm (mM)
Thrombin	S-2238*	100 (0.11)	5	5	40	5	5	60	2	8.3	130	0.20*
	S-2366*	100 (0.14)	5	35	80	70	3	130	2			0.29
FXa	S-2222*	1	100 (0.34)	2	0	2	2	5	1	8.3	130	0.80
	S-2765*	0	100 (0.61)	1	2	1	5	15	1			0.22
FXIa	S-2288*	130	290	100 (0.077)	-	-	-	760	75	8.3	130	1.8
	S-2366*	150	35	100 (0.14)	-	-	-	360	10			2.4
APC	S-2288*	80	30	25	100 (0.13)	15	-	170	-	8.3	-	0.32
	S-2366*	75	4	30	100 (0.19)	60	-	110	-			0.40
Plasmin	S-2251*	4	3	-	2	100 (0.050)	2	-	-	7.4	110	0.80
	S-2302*	15	140	-	40	100 (0.071)	1	-	-			1.0
	S-2366*	80	10	-	70	100 (0.17)	4	-	-			0.80
Single Chain t-PA	S-2288*	170	430	-	-	125	100 (0.058)	1100	-	8.3	130	2.0
	S-2765*	3	590	-	-	15	100 (0.14)	110	-			1.8
									-			
Plasma Kallikrein	S-2288*	35	20	10	25	-	-	100 (0.50)	2			1.2
	S-2302*	3	10	0	5	-	-	100 (0.48)	5			0.44
	S-2366*	20	5	25	40	-	-	100 (0.37)	10			1.2
C1s	S-2765*	10	2300	60	15	-	-	610	100 (0.025)			1.0

B=bovine H=human

Substrate conc. 20 x Km

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< TABLE 1 - SUBSTRATE SELECTIVITY

The selectivity data of the table have been compiled to permit the investigator to understand how a contaminating enzyme would influence the enzyme-substrate reaction under study. Another way of expressing this is to say that the table shows the relative reactivities of two or more enzymes on one particular substrate.

The table should be read horizontally. Each row represents the reactivity of a substrate designated for use with a particular enzyme, indicated to the left, relative to other relevant enzymes.

Example: The set of data in the top row shows the relative reactivity of the thrombin substrate S-2238* with various enzymes. All the experiments were performed using the same buffer, i.e., the one most appropriate for the reaction between thrombin and S-2238*. In addition, the substrate concentration was always the same, or 2 x Km for the reaction of S-2238* with thrombin. The concentrations of the different enzymes are given in Table 2 and are related to the plasma concentration of the corresponding zymogen. The reactivity of S-2238* with thrombin, measured as the time-dependent increase in absorbance (ΔA/min), is given the value 100% (the actual value of ΔA/min is given in brackets). The reactivities of S-2238* with the enzymes FXa, FXIa, APC, plasmin, single chain t-PA, plasma kallikrein, and C1s have then been related to the reactivity of S-2238* with thrombin, and proved to be 5, 5, 40, 5, 5, 60, and 2%, respectively. For information on buffers and Km values, see Table 3.

TABLE 2

Enzyme	Used conc. of enzyme, mg/l	Plasma conc. of zymogen, mg/l
Thrombin	0.040	150
FXa	0.40	8
FXIa	0.20	5.0
APC	0.16	4.0
Plasmin	0.50	200
Single chain t-PA	1.0	0.005
Plasma Kallikrein	0.80	50
C1s	2.0	50

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TABLE 2 - ENZYME CONCENTRATIONS USED IN THE DETERMINATION OF RELATIVE REACTIVITIES

The enzyme concentrations used were in most cases 1/25 of the plasma concentrations of the corresponding zymogens.

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TABLE 3

	K_m (mM)	k_{cat} (1/s)	k_{cat}/K_m (1/(mM•s)) •10^{-3}	Enzyme concentration (mg/l) for ΔA/min=0.05 at [S]=2•K_m
Thrombin, human Buffer: 50 mM Tris HCl, pH 8.3, 130 mM NaCl S-2238* S-2366*	 0.0070 0.15	 180 330	 26 2.2	 0.03 0.02
Thrombin, bovine Buffer: 50 mM Tris HCl, pH 8.3, 130 mM NaCl S-2238* S-2366*	 0.010 0.15	 200 295	 20 2.0	 0.02 0.02
FXa, human Buffer: 50 mM Tris HCl, pH 8.3, 130 mM NaCl, 0.5% BSA S-2222* S-2765*	 1.1 0.60	 100 240	 0.090 0.92	 0.06 0.02
FXa, bovine Buffer: 50 mM Tris HCl, pH 8.3, 130 mM NaCl S-2222* S-2765*	 0.40 0.11	 100 195	 0.25 1.8	 0.06 0.03
FXIa, human Buffer: 50 mM Tris HCl, pH 8.3, 130 mM NaCl, 0.5% BSA S-2288* S-2366*	 0.90 1.2	 190 340	 0.21 0.28	 0.06 0.03
FXIa, bovine Buffer: 50 mM Tris HCl, pH 8.3, 130 mM NaCl, 0.5% BSA S-2288* S-2302* S-2366*	 0.70 0.17 0.73	 21 17 16	 0.030 0.10 0.022	 0.5 0.6 0.7
APC, human Buffer: 100 mM Tris HCl, pH 8.3, 260 mM CsCl, 4mM $CaCl_2$, 0.2% BSA S-2288* S-2366*	 0.16 0.20	 110 190	 0.69 0.95	 0.07 0.04
Plasmin, human Buffer: 50 mM Tris HCl, pH 7.4, 110 mM NaCl S-2251* S-2302* S-2366*	 0.40 0.50 0.40	 20 26 60	 0.050 0.052 0.15	 0.4 0.3 0.1

TABLE 3 - CONT.

	K_m (mM)	k_{cat} (1/s)	k_{cat}/K_m (1/(mM•s)) •10^{-3}	Enzyme concentration (mg/l) for ΔA/min=0.05 at [S]=2•K_m
One-chain t-PA, human Buffer: 50 mM Tris HCl, pH 8.3, 130 mM NaCl, 0.01% Tween 80 S-2288* S-2765*	 1.0 0.9	 10 25	 0.010 0.028	 0.8 0.3
Plasma Kallikrein, human Buffer: 50 mM Tris HCl, pH 7.8, 16mM NaCl (I = 0.05) S-2288* S-2302* S-2366*	 0.60 0.22 0.60	 130 150 80	 0.22 0.68 0.13	 0.1 0.08 0.2
C1s, human Buffer: 50mM Tris HCl, pH 8.0 S-2765*	 0.50	 3	 0.006	 4
Trypsin, bovine Buffer: 50 mM Tris HCl, pH 9.0, 245 mM NaCl S-2222* S-2765*	 0.020 0.010	 240 170	 12 17	 0.01 0.02

^

< TABLE 3 - KINETIC DATA FOR CHROMOGENIC SUBSTRATES AVAILABLE FROM CHROMOGENIX

Suitable chromogenic substrates are listed for a number of serine proteases, most of them part of the cascade systems in blood. Some of the substrates are cleaved by more than one enzyme although at different rates. The kinetic analyses of the enzymatic cleavage of pNA from the substrates were performed under strictly standardized conditions using the clinical chemistry analyser Cobas Mira S. A stable, well-defined temperature is vital for all enzyme kinetic studies, and in this study all reactions were performed at 37 °C. A suitable buffer was chosen for each enzyme, and the pH value given in the compilation is the value to which it was adjusted at 25 °C. Note that the pH value of Tris buffers decreases as the temperature increases, at the rate of approximately 0.1 unit per °C (50 mM Tris-HCl). The kinetics of the reaction was followed spectrophotometrically by measuring the change in absorbance over time, ΔA/min.

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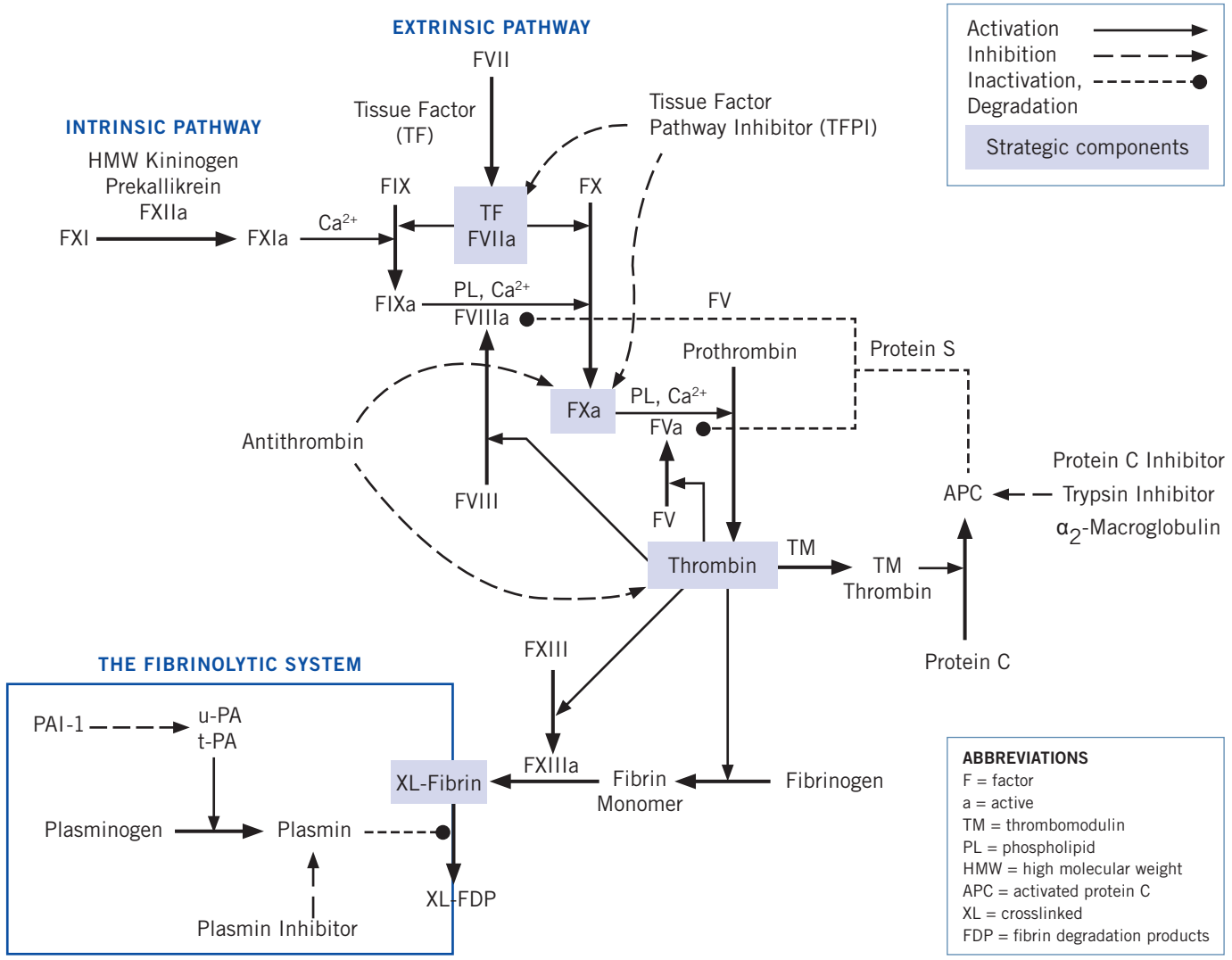
TABLE 4 - PROTEIN CONCENTRATIONS IN PLASMA

Component	Molecular Weight kDa	Plasma Concentration mg/l	Plasma Concentration µmol/l
Fibrinogen	330	3000	9
Prothrombin	72	150	2
Factor V	330	20	0.05
Factor VII	50	0.5	0.01
Factor VIII	330	0.1	0.0003
Factor IX	56	5	0.09
Factor X	59	8	0.13
Factor XI	160	5	0.03
Factor XII	80	30	0.4
Factor XIII	320	10	0.03
Protein C	62	4	0.06
Protein S	70	10(free)	0.14
Protein Z	62	2	0.03
Prekallikrein	86	50	0.6
HMW Kininogen	120	70	0.6
Fibronectin	450	300	0.7
Plasminogen	92	200	2
t-PA	60	0.005	0.0001
Urokinase	53	0.004	0.0001
Antithrombin	58	145	2.5
Heparin Cofactor II	66	80	1.2
Plasmin Inhibitor	63	60	1
Protein C Inhibitor	57	4	0.07
α2-Macroglobulin	725	2000	3

Table 4. Source-Review of current literature.

SCHEMATIC VIEW OF THE HEMOSTATIC SYSTEM – THE COAGULATION CASCADE

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