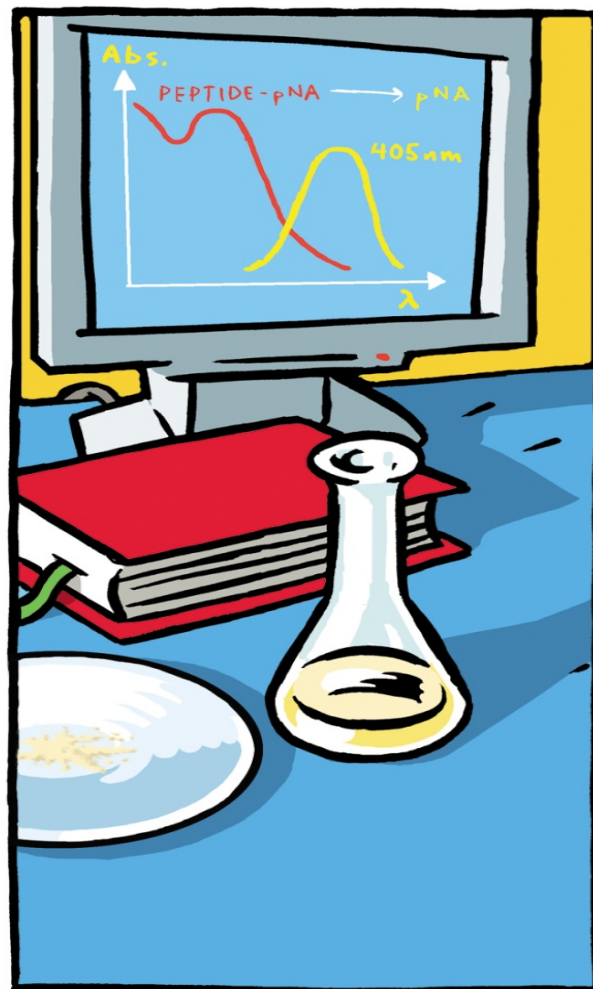




Chromogenic and Fluorogenic Substrates for enzymes involved in Haemostasis



Chromogenic and Fluorogenic Substrates

The PEFACHROME® and PEFAFLUOR® line of chromogenic and fluorogenic peptide substrates is a line of high quality substrates, which allow testing of serine proteinases.

Their focus is on enzymes involved in coagulation and fibrinolysis where the Pentapharm line of substrates carry all the traditional substrates for **thrombin, factor Xa, factor XIIIa, kallikrein, activated protein C, plasmin and plasminogen-SK.**

However, Pentapharm also manufactures substrates for other enzymes such as **collagenase, tryptase, papain, MMP-2, MMP-7, etc.**

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 its catalytic activity is called a substrate.

Chromogenic or fluorogenic substrates are synthetic peptides that react with proteolytic enzymes under formation of colour or fluorescence which can be followed spectrophotometrically and the intensity of which is proportional to the proteolytic activity of the enzyme.

Typically, such substrates are composed of 3 to 5 natural or artificial amino acids. They may be N-terminally protected to reduce undesired degradation by aminopeptidases. On their C-termini they are modified so that upon cleavage of the amide bond a chromogenic or fluorogenic group is released. Detection depends on the type of leaving group and may range from the UV- to the visible region of light (Witt, 1991). Others produce a fluorescent signal.

Most commonly used groups are the **p-nitroaniline** (pNA) which absorbs light of the wavelength of 405 nm and the fluorogenic **7-amino-4-methylcoumarin** (AMC) with an excitation wavelength at 342 nm and an emission wavelength at 440nm.

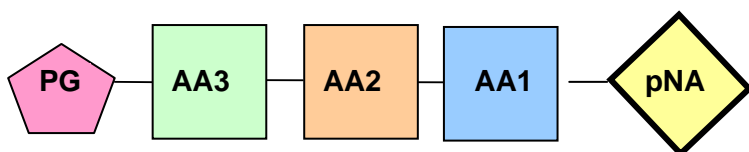


Figure 1: Simplified structure of a chromogenic substrate; PG = protecting group, AA = amino acids, pNA = p-nitroaniline

List of Abbreviations

Aad	α -aminoadipic acid [2-aminohexanedioic acid]
Abu	L- α -aminobutyric acid (2-aminobutyric acid)
AcOH	monoacetate salt
2AcOH	diacetate salt
AMC	7-amino-4-methylcoumarin
β -Ala	β -alanine [3-aminopropionic acid]
But	L- α -aminobutyric acid (2-aminobutyric acid)
Bz	benzoyl
Cbo	benzyloxycarbonyl [Z]
CH ₃ CO	acetyl
CH ₃ SO ₂	methylsulfonyl
CH ₃ OCO	methylloxycarbonyl
CHA	β -cyclohexylalanine [3-cyclohexylalanine]
CHG	cyclohexylglycine
2HCl	hydrogen chloride
MeOSuc	methoxysuccinyl
Nle	L-norleucine [(S)-2-aminocaproic acid]
Nva	L-norvaline [(S)-2-aminopentanoic acid]
PHG	phenylglycine [α -aminophenylacetic acid]
PhPr	3-phenylpropionyl
pNA	para-nitroanilide
Pyr	L-pyrroglutamic acid [(S)-pyrrolidone-(5)-carboxylic acid]
TFA	trifluoroacetate salt
Tos	tosyl [4-toluene sulfonyl]

CHROMOGENIC SUBSTRATES

⇒ If you do not find the substrate you are looking for please contact us at sales-diagnostics@pentapharm.com

Packing sizes: 10 µmol, 25mg, 1 gram and bulk

Pefachrome® TH – series / Chromogenic Substrates for Thrombin

REF	Name	Chemical formula
081-03	Pefachrome® TH	H-D-CHG-Ala-Arg-pNA · 2AcOH
Other thrombin substrates		
081-01	Pefachrome® TH 5244	Tos-Gly-Pro-Arg-pNA · AcOH
081-05	Pefachrome® TH 5247	H-D-CHG-But-Arg-pNA · 2AcOH
081-08	Pefachrome® TH 5250	H-D-CHG-Pro-Arg-pNA · 2AcOH
081-09	Pefachrome® TH 5251	H-D-CHA-Ala-Arg-pNA · 2AcOH
081-11	Pefachrome® 6017	H-D-CHA-Gly-Arg-pNA · 2AcOH
081-15	Pefachrome® TH 5256	CH ₃ OCO-Gly-Pro-Arg-pNA · AcOH
081-17	Pefachrome® TG	H-β-Ala-Gly-Arg-pNA · 2AcOH
081-66	Pefachrome® TH 8198 Comparable to S-2238	H-D-Phe-Pip-Arg-pNA · 2HCl

Pefachrome® FXa – series / Chromogenic Substrates for Factor Xa

REF	Name	Chemical formula
085-06	Pefachrome® FXa	CH₃OCO-D-CHA-Gly-Arg-pNA · AcOH
Other FXa substrates		
085-01	Pefachrome® FXa5277	CH ₃ SO ₂ -D-Leu-Gly-Arg-pNA · AcOH
085-03	Pefachrome® FXa5279	CH ₃ OCO-D-CHG-Gly-Arg-pNA · AcOH
085-05	Pefachrome® FXa5112	C ₂ H ₅ OCO-D-Val-Gly-Arg-pNA · AcOH
085-26	Pefachrome® FXa8592 Comparable to S-2222	Cbo-Ile-Glu(γ-OR)-Gly-Arg-pNA · HCl (R=50% ME, 50% H)
085-27	Pefachrome® FXa8595 Comparable to S-2765	Cbo-D-Arg-Gly-Arg-pNA · 2HCl

Pefachrome® FVIIa – series / Chromogenic Substrates for Factor VII

REF	Name	Chemical formula
093-01	Pefachrome® FVIIa	CH₃SO₂-D-CHA-Abu-Arg-pNA · AcOH

Pefachrome® FIXa – series / Chromogenic Substrates for Factor IXa

REF	Name	Chemical formula
095-02	Pefachrome® FIXa	CH₃SO₂-D-CHG-Gly-Arg-pNA · AcOH

Pefachrome® FXIa – series / Chromogenic Substrates for Factor XIa

REF	Name	Chemical formula
090-41	Pefachrome® FXIa	Z-Aad-Pro-Arg-pNA · AcOH

Pefachrome® FXIIa – series / Chromogenic Substrates for Factor XIIa

REF	Name	Chemical formula
081-11	Pefachrome® 6017	H-D-CHA-Gly-Arg-pNA · 2AcOH

Pefachrome® uPA – series / Chromogenic Substrates for Urokinase

REF	Name	Chemical formula
082-01	Pefachrome® uPA	Bz-β-Ala-Gly-Arg-pNA · AcOH
Other urokinase substrates		
082-05	Pefachrome® uPA8294 Comparable to S-2444	pyroGlu-Gly-Arg-pNA · 2HCl

Pefachrome® tPA – series / Chromogenic Substrates for Tissue Plasminogen Activator

REF	Name	Chemical formula
091-01	Pefachrome® tPA	CH₃SO₂-D-CHA-Gly-Arg-pNA · AcOH
Other substrates for tissue plasminogen activator		
091-03	Pefachrome® tPA5312	CH ₃ SO ₂ -D-Phe-Gly-Arg-pNA · AcOH
091-05	Pefachrome® tPA5314	CH ₃ SO ₂ -D-Abu-Gly-Arg-pNA · AcOH

Pefachrome® PL – series / Chromogenic Substrates for Plasmin

REF	Name	Chemical formula
083-09	Pefachrome® PL5268	H-D-Ala-CHA-Lys-pNA · 2AcOH
Other plasmin substrates		
083-01	Pefachrome® PL5261	Tos-Gly-Pro-Lys-pNA · AcOH
083-02	Pefachrome® PL5262	H-D-But-CHA-Lys-pNA · 2AcOH
083-03	Pefachrome® PL5263	H-D-Nva-CHA-Lys-pNA · 2AcOH
083-04	Pefachrome® PL5264	H-D-Nle-CHA-Lys-pNA · 2AcOH
083-14	Pefachrome® PL5272	H-D-Nle-CHA-Lys-pNA · 2HCl

Pefachrome® GK – series / Chromogenic Substrates for Glandular Kallikrein

REF	Name	Chemical formula
088-01	Pefachrome® GK	H-D-Val-CHA-Arg-pNA · 2AcOH

Pefachrome® PK – series / Chromogenic Substrates for Plasma Kallikrein

REF	Name	Chemical formula
080-03	Pefachrome® PK	H-D-Abu-CHA-Arg-pNA · 2AcOH
Other substrates for Plasma Kallikrein		
080-01	Pefachrome® PK 80714	Bz Pro-Phe-Arg-pNA · AcOH
080-08	Pefachrome® PK 8092 Comparable to S-2302	H-D-Pro-Phe-Arg-pNA · 2HCl

Pefachrome® PCa – series / Chromogenic Substrates for Protein Ca

REF	Name	Chemical formula
089-02	Pefachrome® PCa	H-D-Lys(Cbo)-Pro-Arg-pNA · 2AcOH
Other substrates for activated Protein C		
089-03	Pefachrome® PCa5294	H-D-CHA-Pro-Arg-pNA · 2AcOH
089-04	Pefachrome® PCa5295	H-D-CHA-Pro-Arg-pNA · 2AcOH
089-07	Pefachrome® PCa5297	Pad-Pro-Arg-pNA · AcOH
089-08	Pefachrome® PCa3297	Pyr-CHG-Arg-pNA · AcOH

Pefachrome® C1E – series / Chromogenic Substrates for the Determination of C1-Esterase

REF	Name	Chemical formula
087-03	Pefachrome® C1E	CH₃CO-Lys(Cbo)-Gly-Arg-pNA · AcOH
Other substrates for C1-Esterase		
087-01	Pefachrome® C1E5292	C ₂ H ₅ CO-Lys(Cbo)-Gly-Arg-pNA · AcOH
087-02	Pefachrome® C1E5293	CH ₃ OCO-Lys(Cbo)-Gly-Arg-pNA · AcOH

Pefachrome® TRY – series / Chromogenic Substrates for Trypsin

REF	Name	Chemical formula
084-01	Pefachrome® TRY5274	Cbo-Val-Gly-Arg-pNA · AcOH
Other trypsin substrates		
084-03	Pefachrome® TRY5276	Cbo-Gly-D-Ala-Arg-pNA · AcOH

Pefachrome® LAL – series / Chromogenic Substrates for the Determination of Bacterial Endotoxins

REF	Name	Chemical formula
086-06	Pefachrome® LAL	CH₃SO₂-D-CHA-Gly-Arg-pNA · AcOH
Other substrates for bacterial endotoxins		
086-01	Pefachrome® LAL5288	CH ₃ OCO-D-CHA-Gly-Arg-pNA · AcOH
801741	Pefachrome® LAL Comparable to S-2423	Ac-Ile-Glu-Gly-Arg-pNA.HCl

Other Chromogenic Substrates

REF	Name and chemical formula	Target enzyme
090-08	Pefachrome® ELA MeOSuc-Ala-Ala-Pro-Val-pNA	Leucocyte elastase

Pefachrome®

Comparison of the reactivity of selected chromogenic peptide substrates from Pentapharm

Enzyme	Pefachrome® FXa	Pefachrome® FIXa	Pefachrome® FIXa3960	Pefachrome® FXIa	Pefachrome® FXIa3371	Pefachrome® FXIIa	Pefachrome® PCa	Pefachrome® uPA	Pefachrome® tPA	Pefachrome® Tryp	Pefachrome® PK	Pefachrome® LAL	Pefachrome® ELA	Pefachrome® Tryp	Pefachrome® FVIIa	Pefachrome® GK	Pefachrome® TH	Pefachrome® PL
APC	0.002	0.004	0.037	0.004	0.111	0.006	0.057	0.001	0.002	0.001	0.011	0.003	0.001	0.002	0.022	0.001	0.011	0.001
Factor IXa	0.014	0.054	0.005	0.001	0.005	0.003	0.001	0.001	0.025	0.001	0.001	0.025	0.001	0.001	0.003	0.001	0.001	0.001
Factor VIIa	0.003	0.003	0.005	0.004	0.003	0.002	0.004	0.001	0.004	0.001	0.002	0.005	0.001	0.001	0.009	0	0.001	0
Factor Xa	0.039	0.024	0.002	0.002	0.003	0.016	0.003	0.003	0.046	0.003	0.001	0.039	0.001	0.001	0.003	0	0.003	0
Plasma Kallikrein	0.010	0.014	0.071	0.048	0.047	0.012	0.054	0.001	0.022	0.002	0.073	0.030	0.001	0.001	0.048	0.042	0.027	0.005
Glandular Kallikrein	0.001	0.001	0.002	0.000	0.005	0.001	0.004	0.000	0.001	0.001	0.027	0.001	0.001	0.001	0.001	0.023	0.001	0.001
Factor XIIa	0.019	0.013	0.012	0.004	0.024	0.048	0.013	0.003	0.024	0.006	0.025	0.025	0.001	0.001	0.004	0.014	0.01	0
Factor XIa	0.042	0.030	0.041	0.079	0.060	0.017	0.053	0.003	0.045	0.003	0.003	0.050	0.001	0.007	0.084	0.006	0.053	0.001
Plasmin	0.040	0.039	0.046	0.047	0.140	0.009	0.068	0.002	0.052	0.002	0.058	0.054	0.001	0.080	0.082	0.05	0.026	0.042
Thrombin	0.006	0.027	0.025	0.008	0.001	0.187	0.159	0.001	0.041	0.001	0.002	0.025	0.001	0.021	0.05	0.005	0.185	0.001
tPA	0.021	0.015	0.003	0.001	0.002	0.018	0.003	0.002	0.035	0.003	0.003	0.035	0.001	0.001	0.013	0.001	0.002	0.002
Trypsin	0.095	0.081	0.052	0.019	0.082	0.067	0.057	0.038	0.103	0.025	0.020	0.127	0.001	0.016	0.042	0.02	0.048	0.07
uPA	0.008	0.008	0.007	0.013	0.015	0.008	0.008	0.064	0.005	0.010	0.002	0.005	0.001	0.020	0.006	0.001	0.011	0
Tryptase	0.055	0.004	0.012	0.037	0.020	0.007	0.033	0.004	0.021	0.004	0.002	0.022	0.000	0.045	0.076	0.003	0.016	0.002
Gingipain	0.051	0.032	0.008	0.021	0.011	0.049	0.007	0.043	0.052	0.038	0.016	0.049	0.001	0.001	0.035	0.023	0.034	0.004

The table shows the reactivity given as $\Delta OD/min$ of Pefachrome® Substrates with various proteinases. The optimal choice of substrate for a particular enzyme in terms of sensitivity and selectivity is indicated by bold figures. Determination was done according to the assay protocols in this catalogue.

FLUOROGENIC SUBSTRATES

Packing sizes: 10 µmol, 25mg, 1 gram and bulk

Pefalfluor[®] TH – series / Fluorogenic Substrates for Thrombin

REF	Name	Chemical formula
081-19	Pefalfluor[®] TH	H-D-CHA-Ala-Arg-AMC · 2AcOH

Pefalfluor[®] FXa – series / Fluorogenic Substrates for Factor Xa

REF	Name	Chemical formula
085-12	Pefalfluor[®] FXa	CH₃SO₂-D-CHA-Gly-Arg-AMC · AcOH

Pefalfluor[®] FIXa – series / Fluorogenic Substrates for Factor IXa

REF	Name	Chemical formula
095-03	Pefalfluor[®] FIXa	CH₃SO₂-D-CHG-Gly-Arg-AMC · AcOH
Other substrates for activated F IX		
095-04	Pefalfluor [®] FIXa3688	H-D-Leu-PHG-Arg-AMC · 2AcOH

Pefalfluor[®] uPA – series / Fluorogenic Substrates for Urokinase

REF	Name	Chemical formula
082-03	Pefalfluor[®] uPA	Bz-β-Ala-Gly-Arg-AMC · AcOH
Other urokinase substrates		

Pefalfluor[®] tPA – series / Fluorogenic Substrate for Tissue Plasminogen Activator

REF	Name	Chemical formula
091-06	Pefalfluor[®] tPA	CH₃SO₂-D-Phe-Gly-Arg-AMC · AcOH

Pefalfluor[®] PCa – series / Fluorogenic Substrates for Protein C_a

REF	Name	Chemical formula
089-05	Pefalfluor[®] PCa	Pyr-Pro-Arg-AMC · AcOH
Other substrates for bacterial endotoxins		
089-10	Pefalfluor [®] PCa3342	Pyr-CHG-Arg-AMC · AcOH

Pefafluor® LAL – series / Fluorogenic Substrates for the Determination of Bacterial Endotoxins

REF	Name	Chemical formula
086-04	Pefafluor® LAL5291	CH ₃ SO ₂ -D-CHA-Gly-Arg-AMC · AcOH

Pefafluor® MMP – series / Fluorogenic Substrates for Matrix Metalloproteinases

REF	Name	Chemical formula
800049	Pefafluor® MMP-2 Abz-Lys-Leu-Pro-Ala-Nva-Glu(pNA)- Ala-Arg-NH ₂ · 3TFA	Matrix metalloproteinase 2
800050	Pefafluor® MMP-7 Abz-Lys-Pro-Leu-Glu-Leu-Glu(pNA)-Ala Arg-NH ₂ · 3TFA	Matrix metalloproteinase 7



Snake venom enzymes by Pentapharm



Natural Activators

Coagulation factors in plasma are usually inactive and require a proteolytic activation as the first step in a clotting or chromogenic assay. It is often an advantage to use highly purified and specific snake venom enzymes to activate coagulation factors rather than using other non-physiological or physiological activators. In contrast to other activators, many snake venom enzymes are not dependent on cofactors, phospholipids or calcium ions.

This allows the development of relatively simple and highly specific assays.

Features

- Highly specific biocatalysts

To be used in coagulation and platelet aggregation tests, in photometric assays as well as in immunological systems for:

- research
- diagnostic purposes
- analytical applications
- quality control

Highly purified proteins, isolated from snake venoms for use as specific activators of serine proteases and for platelet research

Activator	Source	Application	Remarks
Convulxin REF: 119-02	Crotalus durissus	Activation of human platelets via binding and clustering of GPVI-receptors under physiological conditions	
Ecarin REF: 116-01	Echis carinatus	Prothrombin assay Determination of hirudin	Metalloprotease, activates specifically prothrombin via meizothrombin, does not need PL and Ca ²⁺
Protac [®] REF 113-01 REF 113-04	Agkistrodon contortrix	Determination of protein C and protein S Protein C pathway assays	Serine protease
Pefakit[®] Reptilase[®] Time REF: 800191	Bothrops atrox	Assay for measurement of functionality of fibrin polymerisation afibrinogenemia or hypofibrinogen-emia hyperfibrinolysis thrombolytic therapy	CE-marked IVD Serine protease, does not affect other coagulation factors than fibrinogen, cleaves only FPA
Rhodocytin On request	Calloselas marhodostoma	Rhodocytin Induces Platelet Aggregation by Interacting with Glycoprotein Ia/IIa (GPIa/IIa, Integrin $\alpha_2\beta_1$)	Ligand for the platelet-activating receptor CLEC-2
RVV-V REF: 121-03	Daboia (Vipera) russelli	Activation of FV in APC-R assay PiCT [®] (assay for heparin, LMWH and other anticoagulants)	Serine protease, not inhibited by AT
RVV-X REF: 121-07 REF: 121-06	Daboia (Vipera) russelli	Determination of FX Screening assay for lupus anti-coagulants (diluted Russel viper venom test, dRVVT)	Metalloprotease, does also (weakly) activate protein C and factor IX

Research and Diagnostic Use of Purified Snake Venom Enzymes

The hemostatic system of vertebrates is an important target for snake venoms. It bases on a vulnerable interaction - easily influenced by biochemical and biophysical factors - between thrombocytes, endothelial cells, subendothelial structures and plasma proteins. Disturbance of this sensitive interaction can lead to life-threatening thromboembolic events or to fatal blood loss. Venomous snakes have co-evolved with their prey animals and have developed a variety of proteins and enzymes in their venoms which specifically interact with components of the hemostatic system of their prey. Individual snake venom components often act in the same way as the corresponding physiological clotting factors, with the essential difference that the prey organism often does not have the regulating inhibitors counteracting the activators from snake venom.

Although this property can be fatal for the prey, some isolated snake venom components are highly interesting for diagnostic, research or therapeutic purposes (Stocker, 1990).

Being natural products, snake venoms have some variability in their composition. Therefore, the batches have to be standardized in appropriate assays. For many applications, fractions are isolated from the snake venom by adequate purification steps until homogeneity of the enzyme. This is of particular importance when enzymes with counteracting actions are found in the snake venom. In the hemostasis laboratory, purified snake venom components are used in various clotting assays and partly also in chromogenic substrate methods.