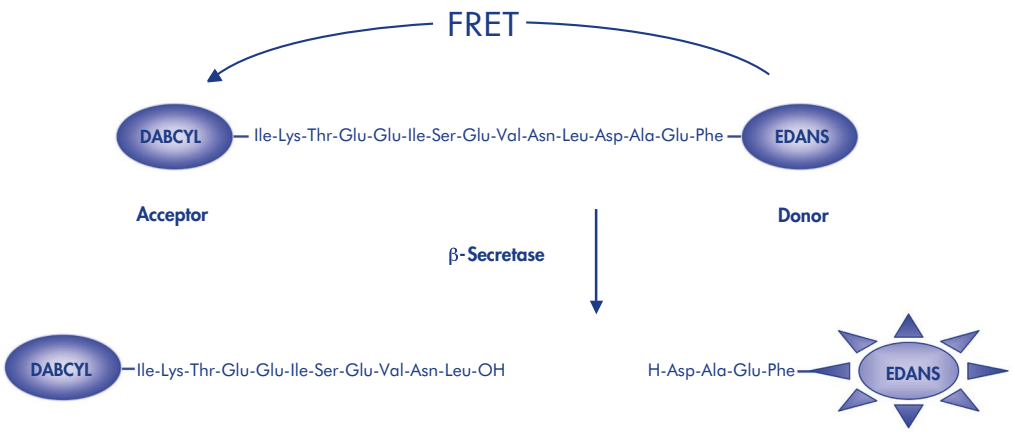
interchim

BACHEM

Product Monograph

FRET Substrates



Abstract

Fluorescence Resonance Energy Transfer (FRET) is the non-radiative transfer of energy from an excited fluorophore (or donor) to a suitable quencher (or acceptor) molecule. The energy transfer is influenced by the spectral overlap of the donor and acceptor molecules, their distance from each other and the relative orientation of the donor and acceptor transition dipoles.
The physical principle of FRET is used in a variety of applications including the measurement of protease activity of substrates, with the fluorophore separated from the quencher by a short peptide sequence containing the enzyme cleavage site. The spatial separation of the donor from the acceptor molecule by proteolysis of the peptide bond results in an increase in fluorescence because the energy transfer efficiency decreases exponentially with the separating distance.
In this monograph Bachem presents a range of highly sensitive FRET protease substrates for a variety of enzymes.

Introduction

Fluorophores are substances which, like chromophores, absorb light in the UV or visible range. In contrast to chromophores they re-emit part of the light as radiation. This process is called fluorescence and can be illustrated by the energy level diagram suggested by A. Jablonski.
Absorption of light ($h\nu_A$) causes an electron to be promoted from its electronic ground state (designated as S_0) to an excited state (usually S_1). Every energy state has several vibrational energy levels 0, 1, 2 etc.. During the lifetime of the excited state, i.e. the time elapsed between excitation of the molecule and emission of the photon (usually between 1-10 ns) part of the energy is lost by internal vibration. As a result the wavelength of the emitted light ($h\nu_F$) is always longer than that of the exciting light. This phenomenon is called the Stokes shift and allows the detection of emission against a background of light derived from excitation. Usually, the fluorescence excitation spectrum of a fluorophore in a diluted solution is identical to its absorption spectrum and under the same conditions, the fluorescence emission spectrum is independent of the excitation wavelength.

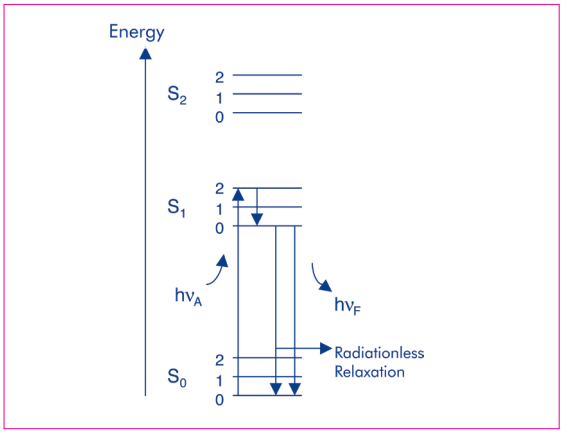


Fig. 1:
Energy Level Diagram

In a diluted solution, fluorescence intensity is linearly proportional to several parameters as deduced from Lambert-Beer's law. These are the molar absorption coefficient, the path length, the intensity of the incident light, and the quantum yield which is the ratio of the number of emitted to the total number of absorbed photons.
Fluorescence detection is dependent on the sensitivity of the instrument and is therefore measured in arbitrary units. Higher concentrations of the fluorophore (> 0.1 absorption units) lead to deviations from the linearity due to loss of excitation intensity across the cuvette path length as the excitation light is absorbed by the fluorophore. This phenomenon is known as the inner filter effect. Other effects which influence fluorescence measurements are related to intrinsic or background fluorescence originating from sample preparations and buffer contaminants, respectively. To minimize fluorescence derived from contaminants it is recommended to use materials of the highest purity.
Fluorescence spectra may also be dependent on the solvent. With some fluorophores such as 2-acetylanthracene or tryptophan a spectral shift to longer wavelengths (bathochromic shift or red shift) is observed in more polar solvents. As mentioned with AMC (please see below) the pH of a solution might also change the fluorescence properties of a fluorophore.

Fluorescence Quenching

Any process which decreases the fluorescence intensity of a given substance can be referred to as quenching. Several types of quenching processes can be distinguished. These include collisional and static quenching, as well as fluorescence resonance energy transfer (FRET). Collisional or dynamic quenching can be considered as a reduction in fluorescence intensity due to a collision of the quencher with the fluorophore in the excited state. Upon contact the fluorophore returns to the ground state without light emission. One of the best known collisional quenchers which quenches almost all known fluorophores is molecular oxygen. It is therefore often required to remove dissolved oxygen to obtain reliable measurements. In static quenching a non-fluorescent complex is formed between the quencher and the fluorophore. In contrast to both of these quenching processes, FRET does not require contact of the quencher with the fluorophore. The energy transfer occurs without the appearance of a photon.

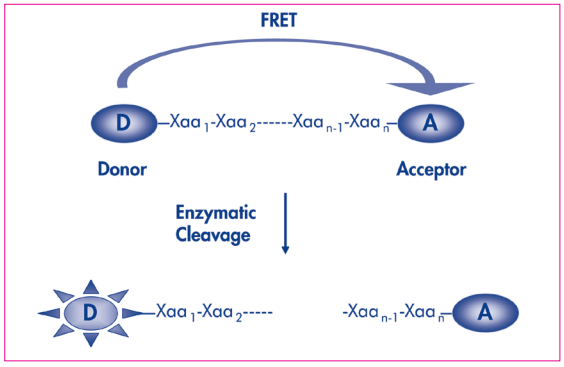


Fig. 2: Fluorescence Resonance Energy Transfer (FRET)

Fluorescence Resonance Energy Transfer (FRET)

Fluorescence resonance energy transfer (FRET) is the transfer of the excited state energy of a donor to an acceptor without the emission of light. The energy transfer can be considered as an energy exchange of an oscillating dipole to a dipole with similar resonance frequency. FRET can only take place when the emission spectrum of the donor overlaps with the absorption spectrum of the acceptor. The donor and acceptor have to be within a distance of 1-10 nm. The energy transfer efficiency depends on the extent of the overlap of the emission spectrum of the donor with the absorption spectrum of the acceptor, the relative orientation of the donor and acceptor transition dipoles, and the distance r between donor and acceptor. The energy transfer efficiency decreases exponentially by r^6. The distance at which the efficiency of energy transfer is reduced by 50 % is a characteristic value for a given donor acceptor pair and is called the Förster distance R_0.

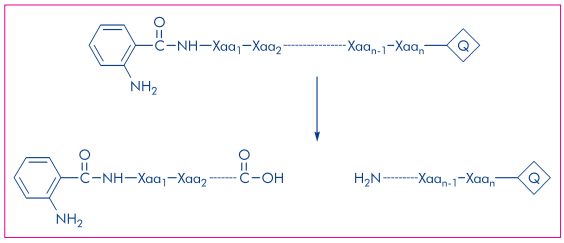
Fluorophore	Excitation Wavelength*	Emission Wavelength	References
Abz (2-Aminobenzoyl or Anthraniloyl)	320 nm	420 nm	[1], [2], [3]
N-Me-Abz (N-Methyl-anthraniloyl)	340-360 nm	440-450 nm	[4]
Dansyl (5-(Dimethylamino)naphthalene-1-sulfonyl)	342 nm	562 nm	[5]
EDANS (5-[(2-Aminoethyl)amino]naphthalene-1-sulfonic acid)	340 nm	490 nm	[6]
FITC (Fluorescein isothiocyanate)	490 nm	520 nm	[7]
Lucifer Yellow (6-Amino-2,3-dihydro-1,3-dioxo-2-hydrazinocarbonyl-amino-1H-benz[d,e]isoquinoline-5,8-disulfonic acid)	430 nm	520 nm	[8]
Mca ((7-Methoxycoumarin-4-yl)acetyl)	325 nm	392 nm	[9]
Trp (Tryptophan)	280 nm	360 nm	[1]

* the values listed are as reported in the cited literature.

Table 1: Fluorophores

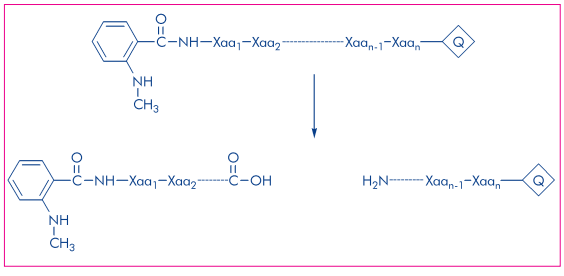
Abz (2-Aminobenzoyl or Anthraniloyl) Substrates

Abz substrates are generally used in connection with a number of quenchers (Q) such as Dnp (2,4-dinitrophenyl), EDDnp (N-(2,4-dinitrophenyl)ethylenediamine) or 4-nitro-phenylalanine and 3-nitro-tyrosine. Substrate cleavage can be detected at 420 nm using an excitation wavelength of 320 nm.



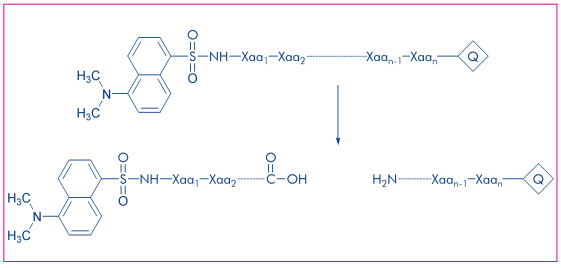
N-Me-Abz (N-Methyl-anthraniloyl) Substrates

N-Me-Abz substrates are generally used with Dnp as quencher (Q). The fluorescent group is either linked to the amino-terminal amino group or the ε-amino group of a lysine residue. Substrate cleavage can be detected at 440-450 nm using an excitation wavelength of 340-360 nm.



Dansyl (5-(Dimethylamino)naphthalene-1-sulfonyl) Substrates

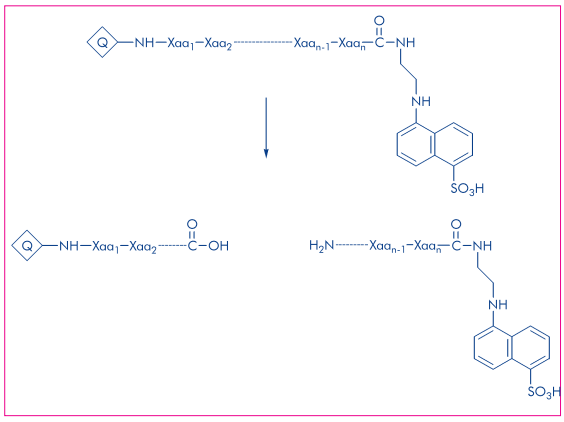
In a few substrates the fluorescent dansyl group serves as donor with 4-nitro-phenylalanine as acceptor (Q). Substrate cleavage can be assayed at 562 nm using excitation at 342 nm. More commonly the Dansyl group is used as a quencher for tryptophan fluorescence.



EDANS (5-[(2-Aminoethyl)amino]naphthalene-1-sulfonic acid) Substrates

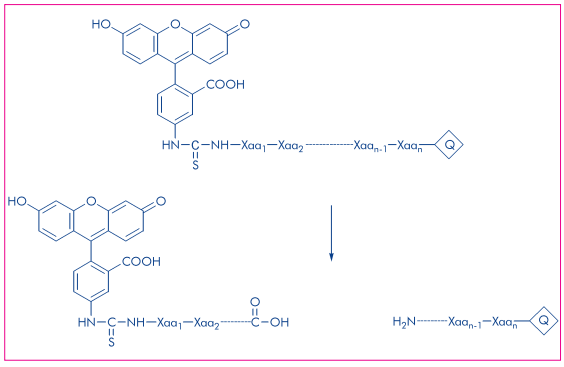
In these substrates, the fluorescence of the EDANS group is generally quenched by the DABCYL (4-(4-dimethyl-aminophenylazo)benzoyl) group (Q).
The DABCYL group is usually conjugated to the N-terminus and the EDANS group ($\varepsilon_{305\ nm}$ = 24700 $M^{-1}cm^{-1}$) attached to the C-terminus of the peptide substrate.

Substrate cleavage can be detected at 490 nm using an excitation wavelength of 340 nm.



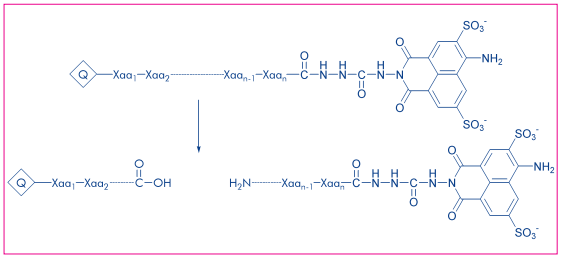
FITC (Fluorescein isothicyanate) Substrates

Only few FITC substrates have been described. The FITC label can be quenched with Dnp (Q). Substrate cleavage can be detected at 520 nm using an excitation wavelength of 490 nm.



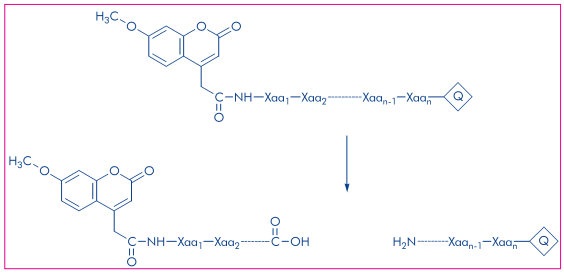
Lucifer Yellow (6-Amino-2,3-dihydro-1,3-dioxo-2-hydrazinocarbonylamino-1H-benz[d,e]isoquinoline-5,8-disulfonic acid) Substrates

Lucifer Yellow can be detected at 520 nm using excitation at 430 nm. It is efficiently quenched by Dabsyl (4-(4-Dimethylaminophenylazo)-benzenesulfonyl) (Q).



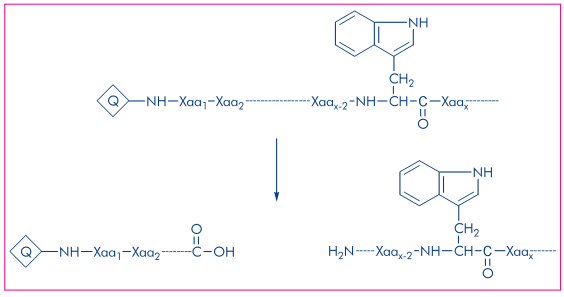
Mca ((7-Methoxycoumarin-4-yl)acetyl) Substrates

In this kind of substrates Mca is bound to an amino group (usually the N-terminal amino group) of a peptide sequence and quenched by Dnp (Q). The cleaved peptide fragment with the attached Mca group can be detected fluorometrically at 392 nm using an excitation wavelength of 325 nm.



Trp (Tryptophan) Substrates

Tryptophan (like tyrosine and phenylalanine) is a fluorescent amino acid which has been used in a variety of substrates with Dnp as a quencher (Q). Substrate cleavage can be detected at 360 nm using an excitation wavelength of 280 nm.



Below you can find a list of common donor acceptor pairs used for the design of FRET enzyme substrates.

Donor (Fluorphore)	Acceptor (Quencher)	References
Abz (2-Aminobenzoyl or Anthraniloyl)	**Dnp** (2,4-Dinitrophenyl)	[1]
Abz (2-Aminobenzoyl or Anthraniloyl)	**EDDnp** (N-(2,4-Dinitrophenyl)ethylenediamine)	[10]
Abz (2-Aminobenzoyl or Anthraniloyl)	**4-Nitro-Phe** (4-Nitro-phenylalanine)	[11]
Abz (2-Aminobenzoyl or Anthraniloyl)	**3-Nitro-Tyr** (3-Nitro-tyrosine)	[12]
N-Me-Abz (N-Methyl-anthraniloyl)	**Dnp** (2,4-Dinitrophenyl)	[4]
Dansyl (5-(Dimethylamino)naphthalene-1-sulfonyl)	**4-Nitro-Phe** (4-Nitro-phenylalanine)	[5]
EDANS (5-[(2-Aminoethyl)amino]-naphthalene-1-sulfonic acid)	**DABCYL** (4-(4-Dimethylaminophenylazo)benzoyl)	[6]
FITC (Fluorescein isothiocyanate)	**Dnp** (2,4-Dinitrophenyl)	[13]
Lucifer Yellow (6-Amino-2,3-dihydro-1,3-dioxo-2-hydrazinocarbonylamino-1H-benz[d,e]isoquinoline-5,8-disulfonic acid)	**Dabsyl** (4-(4-Dimethylaminophenylazo)-benzenesulfonyl)	[8]
Mca ((7-Methoxycoumarin-4-yl)acetyl)	**Dnp** (2,4-Dinitrophenyl)	[9]
Trp (Tryptophan)	**Dnp** (2,4-Dinitrophenyl)	[1]
Trp (Tryptophan)	**4-Nitro-Z** (4-Nitro-benzyloxycarbonyl)	[14]

Table 2:
Donor/Acceptor Pairs

References

[1] M.H. Cezari et al.
Cathepsin B carboxydipeptidase specificity analysis using internally quenched fluorescent peptides.
Biochem. J. **368**, 365-369 (2002)

[2] L. Bourgeois et al.
Serpin-derived peptide substrates for investigating the substrate specificity of human tissue kallikreins hK1 and hK2.
J. Biol. Chem. **272**, 29590-29595 (1997)

[3] K.N. Parameswaran et al.
Hydrolysis of gamma:epsilon isopeptides by cytosolic transglutaminases and by coagulation factor XIIIa.
J. Biol. Chem. **272**, 10311-10317 (1997)

[4] D.M. Bickett et al.
A high throughput fluorogenic substrate for interstitial collagenase (MMP-1) and gelatinase (MMP-9).
Anal. Biochem. **212**, 58-64 (1993)

[5] D. Florentin et al.
A highly sensitive fluorometric assay for „enkephalinase," a neutral metalloendopeptidase that releases tyrosine-glycine-glycine from enkephalins.
Anal. Biochem. **141**, 62-69 (1984)

[6] E.D. Matayoshi et al.
Novel fluorogenic substrates for assaying retroviral proteases by resonance energy transfer.
Science **247**, 954-958 (1990)

[7] A. Chersi et al.
Preparation and utilization of fluorescent synthetic peptides.
Biochim. Biophys. Acta **1034**, 333-336 (1990)

[8] F. Grüninger-Leitch et al.
Substrate and inhibitor profile of BACE (beta-secretase) and comparison with other mammalian aspartic proteases.
J. Biol. Chem. **277**, 4687-4693 (2002)

[9] T. Kondo et al.
Activation of distinct caspase-like proteases by Fas and reaper in Drosophila cells.
Proc. Natl. Acad. Sci. USA **94**, 11951-11956 (1997)

[10] D. Andrau et al.
BACE1- and BACE2-expressing human cells: characterization of beta-amyloid precursor protein derived catabolites, design of a novel fluorimetric assay, and identification of new in vitro inhibitors.
J. Biol. Chem. **278**, 25859-25866 (2003)

[11] M.V. Toth and G.R. Marshall
A simple, continuous fluorometric assay for HIV protease.
Int. J. Peptide Protein Res. **36**, 544-550 (1990)

[12] K. Breddam and M. Meldal
Substrate preferences of glutamic-acid-specific endopeptidases assessed by synthetic peptide substrates based on intramolecular fluorescence quenching.
Eur. J. Biochem. **206**, 103-107 (1992)

[13] H.J. Korting et al.
Fluorometric determination of the quality of FITC conjugates.
Virologie **28**, 41-43 (1977)

[14] A. Persson and I.B. Wilson
A fluorogenic substrate for angiotensin-converting enzyme.
Anal. Biochem. **83**, 296-303 (1977)

For further details, please see the following literature references

J. Bergmeyer
Methods of Enzymatic Analysis, 3rd Edition, Vol. I, Fundamentals
J. Bergmeyer and M. Grassl, eds., Verlag Chemie GmbH, Weinheim (1983)

J. Bergmeyer
Methods of Enzymatic Analysis, 3rd Edition, Vol. II, Samples, Reagents, Assessment of Results
J. Bergmeyer and M. Grassl, eds., Verlag Chemie GmbH, Weinheim (1983)

J.R. Lakowicz
Principles of Fluorescence Spectroscopy
Plenum Press, New York (1983)

Prod.No. Product

ACE Substrates

M-1100 **Abz-Gly-p-nitro-Phe-Pro-OH**
Trifluoroacetate salt
Fluorogenic substrate for angiotensin I-converting enzyme.
Lit. A. Carmel and A. Yaron, Eur. J. Biochem. ***87****, 265 (1978)/ A. Carmel et al., Clin. Chim. Acta* ***93****, 215 (1979)/ A. Yaron et al., Anal. Biochem.* ***95****, 228 (1979)/ H.M. Neels et al., Clin. Chim. Acta* ***141****, 281 (1984)/ W. Raasch et al., J. Hypertens.* ***20****, 2495 (2002)*
Solubility: 50 mg/ml in methanol

M-2590 **Abz-Phe-Arg-Lys(Dnp)-Pro-OH**
Hydrochloride salt
Excellent angiotensin I-converting enzyme (ACE) substrate with a Km value of 4.0 µM and a kcat value of 210 s^{-1}.
Lit. M.C. Araujo et al., Biochemistry ***39****, 8519 (2000)/ D.A. Elswijk et al., J. Chromatogr. A.* ***1020****, 45 (2003)*
Solubility: in 0.1 % TFA in acetonitrile/water

ACE2 Substrates

M-2660 **Abz-Ser-Pro-3-nitro-Tyr-OH**
Selective substrate for angiotensin-converting enzyme 2 (ACE2), a novel ACE homolog, which differs in its specificity and physiological role from ACE. The internally quenched fluorescent substrate is potentially useful in applications such as high-throughput screening of ACE2 inhibitors (Km = 23 µM, kcat/Km = $3.5 \cdot 10^4$ $M^{-1}s^{-1}$).
Lit. Z.-H. Yan et al., Anal. Biochem. ***312****, 141 (2003)/ F.J. Warner et al., Cell. Mol. Life Sci.* ***61****, 2704 (2004)*
Solubility: at least 1 mg/ml in DMSO or 20 % acetic acid

Cathepsin Substrates

M-2595 **Abz-Gly-Ile-Val-Arg-Ala-Lys(Dnp)-OH**
Very efficient and selective FRET substrate for human cathepsin B (Km = 5.9 µM, kcat = 43 s^{-1}, kcat/Km = 7288 $mM^{-1}s^{-1}$). The kcat/Km values for cathepsin K, L, V, X, and cruzain were 133.3, 100, 32, 17, and 75 $mM^{-1}s^{-1}$, respectively.
Lit. S.S. Cotrin et al., Anal. Biochem. ***335****, 244 (2004)*
Solubility: 1 mg/ml in 50 % acetic acid

M-2600 **Abz-Glu-Ile-Phe-Val-Phe-Lys-Gln-EDDnp**
Trifluoroacetate salt
(Abz-EIFVFKQ-EDDnp)
This fluorescence resonance energy transfer (FRET) peptide is a useful substrate for cathepsin P, a recently discovered placental cysteine protease that is structurally related to the more ubiquitously expressed broad-specificity enzyme cathepsin L.
Lit. L. Puzer et al., Arch. Biochem. Biophys. ***435****, 190 (2005)*
Solubility: 1 mg/ml in 80 % acetic acid

Prod.No. Product

Cytomegalovirus (CMV) Protease Substrates

M-2450 **Abz-tBu-Gly-tBu-Gly-Asn(Me)$_2$-Ala-Ser-Ser-Arg-Leu-3-nitro-Tyr-Arg-OH**
Trifluoroacetate salt
Improved fluorogenic substrate for the determination of human cytomegalovirus protease. It displayed a kcat/Km value of 15940 $M^{-1}s^{-1}$, i.e., more than 60-fold greater than that of the equivalent, non-optimized substrate Abz-Val-Val-Asn-Ala-Ser-Ser-Arg-Leu-3-nitro-Tyr-Arg-OH under identical conditions.
Lit. P.R. Bonneau et al., Anal. Biochem. ***255****, 59 (1998)*
Solubility: 1 mg/ml in water

Furin Substrates

M-2115 **Abz-Arg-Val-Lys-Arg-Gly-Leu-Ala-m-nitro-Tyr-Asp-OH**
Trifluoroacetate salt
This internally quenched fluorogenic peptide substrate contains anthranilic acid as fluorescent donor and m-nitro-tyrosine as acceptor (quencher). Its sequence is based on the sequence of hemagglutinin. This substrate is efficiently cleaved by furin, a subtilisin-like eukaryotic serine endo-protease with Km = 3.8 µM and kcat = 29.3 s^{-1} (kcat/Km = 7710000 $M^{-1}s^{-1}$). Its kcat/Km value is over 2000-fold higher than that of the commonly used substrate Boc-Arg-Val-Arg-Arg-AMC (I-1645).
Lit. H. Angliker et al., Anal. Biochem. ***224****, 409 (1995)*
Solubility: in water

Galanin Degrading Zn-Metallopeptidase Substrates

M-2365 **(Abz-Gly^1)-Galanin (1-10)-Lys(retro-m-nitro-Tyr-H) amide (human)**
Abz-Gly-Trp-Thr-Leu-Asn-Ser-Ala-Gly-Tyr-Leu-Lys(retro-m-nitro-Tyr-H)-NH_2
Trifluoroacetate salt
Substrate for the detection of a galanin degrading 70 kD Zn-metallopeptidase from bovine spinal cord. The value of Km was calculated to 72.1 µM and v_{max} to 18.2 µM/min.
Lit. A. Juréus et al., Neuropeptides ***32****, 453 (1998)*
Solubility: 5 mg/ml in methanol

* Q = Dnp, EDDnp, p-nitro-Phe, m-nitro-Tyr, p-Nitrobenzylamide

Prod.No. Product

HIV Protease Substrates

H-2992 **Anthranilyl-HIV Protease Substrate**
Abz-Thr-Ile-Nle-p-nitro-Phe-Gln-Arg-NH_2
Trifluoroacetate salt
This fluorogenic hexapeptide substrate is derived from the p24/p15 cleavage site of the viral gag-pol poly-protein. A simple, continuous fluorometric assay for HIV protease has been developed, which allows the screening of potential HIV protease inhibitors.
Lit. M.V. Toth and G.R. Marshall, Int. J. Peptide Protein Res. ***36****, 544 (1990)*
Solubility: at least 1 mg/ml in DMSO or 20 % acetic acid with agitation

H-1044 **Anthranilyl-HIV Protease Substrate III**
Abz-His-Lys-Ala-Arg-Val-Leu-p-nitro-Phe-Glu-Ala-Nle-Ser-NH_2
Trifluoroacetate salt
Solubility: in water

H-1052 **Anthranilyl-HIV Protease Substrate IV**
Abz-Lys-Ala-Arg-Val-Nle-p-nitro-Phe-Glu-Ala-Nle-NH_2
Trifluoroacetate salt
Solubility: at least 1 mg/ml in 0.1 % TFA with agitation

H-1168 **Anthranilyl-HIV Protease Substrate V**
Abz-Ala-Arg-Val-Nle-p-nitro-Phe-Glu-Ala-Nle-NH_2
Trifluoroacetate salt
Solubility: at least 1mg/ml in DMSO with agitation

H-1204 **Anthranilyl-HIV Protease Substrate VI**
Abz-Arg-Val-Nle-p-nitro-Phe-Glu-Ala-Nle-NH_2
Trifluoroacetate salt
Hydrolysis of the anthranilyl fluorogenic substrates is monitored by the decrease in fluorescence quenching upon separation of the anthranilyl chromophore from the p-nitro-Phe quencher.
Lit. B.M. Dunn et al., Poster presented at the 12th American Peptide Symposium, Cambridge, MA (1991)/ M.W. Pennington et al., Peptides 1992, Proceedings of the 22nd European Peptide Symposium, Interlaken, Switzerland, p. 936, C.H. Schneider and A.N. Eberle, eds., Escom, Leiden (1993)
Solubility: in methanol

Human Rhinovirus-14 (HRV14) 2A Protease Substrates

M-2360 **H-Thr-Arg-Pro-Ile-Ile-Thr-Thr-m-nitro-Tyr-Gly-Pro-Ser-Asp-Lys(Abz)-Tyr-OH**
Trifluoroacetate salt
This fluorogenic substrate containing an anthraniloyl group and a 3-nitrotyrosine as the resonance energy transfer donor/quencher pair, was developed for the assay of 2A protease from human rhinovirus (kcat/Km = 154 $M^{-1}s^{-1}$), which has been viewed as an important enzyme target for antiviral intervention.
Lit. Q.M. Wang et al., Arch. Biochem. Biophys. ***356****, 12 (1998)*
Solubility: at least 1 mg/ml in 0.1 % TFA with agitation

Human Rhinovirus-14 (HRV14) 3C Protease Substrates

M-2075 **Abz-Glu-Thr-Leu-Phe-Gln-Gly-Pro-Val-p-nitro-Phe-NH_2**
Trifluoroacetate salt
This peptide corresponds to the 2C-3A cleavage site of the HRV type 14 protein. It is a substrate for the HRV 3C protease *in vitro*.
Lit. M.G. Cordingley et al., J. Biol. Chem. ***265****, 9062 (1990)*
Solubility: at least 1 mg/ml in N-methyl-2-pyrrolidone with agitation

Kallikrein Substrates

M-2665 **Abz-Ala-Phe-Arg-Phe-Ser-Gln-EDDnp**
Trifluoroacetate salt
Best human kallikrein 6 (hK6) FRET substrate so far described (kcat = 11.6 s^{-1}, Km = 0.3 µM, kcat/Km = 38667 $mM^{-1}s^{-1}$).
Lit. P.F. Angelo et al., J. Biol. Chem. ***281****, 3116 (2006)*
Solubility: in 0.1 % TFA in acetonitrile/water

Papain Substrates

M-2100 **Abz-Gln-Val-Val-Ala-Gly-Ala-EDDnp**
Trifluoroacetate salt
This fluorogenic substrate of papain, based on the highly conserved sequence QVVAG of the cystatin family of natural inhibitors, is among the most sensitive papain substrates ever reported (kcat/Km = $29 \cdot 10^6$ $M^{-1}s^{-1}$).
Lit. C. Serveau et al., Biochimie ***76****, 153 (1994)*
Solubility: 1 mg/ml in 80 % acetic acid

* Q = Dnp, EDDnp, p-nitro-Phe, m-nitro-Tyr, p-Nitrobenzylamide

Prod.No. Product

β-Secretase Substrates

M-2560 **Abz-Amyloid β/A4 Protein Precursor$_{770}$ (669-674)-EDDnp**
Abz-Val-Lys-Met-Asp-Ala-Glu-EDDnp
(JMV2235; Abz-APP$_{770}$ (669-674)-EDDnp)
Trifluoroacetate salt
Novel intramolecularly quenched fluorescent substrate containing the Abz/EDDnp groups as the donor/acceptor pair. It mimicks the wild-type (JMV2235) β-amyloid precursor protein (βAPP) sequence targeted by β-secretase BACE (β-site APP-cleaving activity). This substrate is cleaved by BACE1, BACE2, and cathepsin D.
Lit. D. Andrau et al., J. Biol. Chem. ***278****, 25859 (2003)*
Solubility: at least 1 mg/ml in DMSO or 50 % acetic acid with agitation

M-2565 **Abz-(Asn^{670},Leu^{671})-Amyloid β/A4 Protein Precursor$_{770}$ (669-674)-EDDnp**
Abz-Val-Asn-Leu-Asp-Ala-Glu-EDDnp
(JMV2236; Abz-(Asn^{670},Leu^{671})-APP$_{770}$ (669-674)-EDDnp)
Trifluoroacetate salt
Novel intramolecularly quenched fluorescent substrate containing the Abz/EDDnp groups as the donor/acceptor pair. It corresponds to the Swedish-mutated (JMV2236) β-amyloid precursor protein (βAPP) sequence targeted by β-secretase BACE (β-site APP-cleaving activity). This substrate is more selectively cleaved by BACE1 and BACE2 than by cathepsin D, a disintegrin and metalloprotease 10 (ADAM10), tumor necrosis α-converting enzyme (TACE), presenilin-1 (PS1), or presenilin-2 (PS2).
Lit. D. Andrau et al., J. Biol. Chem. ***278****, 25859 (2003)*
Solubility: at least 1 mg/ml in DMSO with agitation

γ–Secretase Substrates

M-2540 **Abz-Amyloid β/A4 Protein Precursor$_{770}$ (708-715)-Lys(Dnp)-D-Arg-D-Arg-D-Arg amide**
Abz-Gly-Gly-Val-Val-Ile-Ala-Thr-Val-Lys(Dnp)-D-Arg-D-Arg-D-Arg-NH$_2$
(Abz-APP$_{770}$ (708-715)-Lys(Dnp)-D-Arg-D-Arg-D-Arg amide; Abz-Amyloid β-Protein (37-42)-Thr-Val-Lys(Dnp)-D-Arg-D-Arg-D-Arg amide)
Trifluoroacetate salt
Novel sensitive fluorogenic substrate developed for the analysis of γ-secretase from post mortem non-Alzheimer's and Alzheimer's disease human brain isolates.
Lit. M.R. Farmery et al., J. Biol. Chem. ***278****, 24277 (2003)*
Solubility: at least 1 mg/ml in water with agitation

Prod.No. Product

Miscellaneous Substrates

H-6675 **Abz-Ala-Gly-Leu-Ala-p-nitrobenzylamide**
A fluorogenic substrate for neutral metalloendopeptidases, e.g. *Pseudomonas aeruginosa* elastase, enkephalinase (NEP 24.11), and thermolysin.
Lit. N. Nishino and J.C. Powers, J. Biol. Chem. ***255****, 3482 (1980)/ R.S. Rush et al., Arch. Biochem. Biophys.* ***231****, 390 (1984)/ D.I. Mundy and W.J. Strittmatter, Cell* ***40****, 645 (1985)/ H.C. Rempel and L. Pulliam, AIDS* ***19****, 127 (2005)*
Solubility: 10 mg/ml in 50 % acetic acid

M-2475 **Abz-Ala-Phe-Ala-Phe-Asp-Val-Phe-3-nitro-Tyr-Asp-OH**
Trifluoroacetate salt
Fluorometric substrate for Asp-specific proteases from *Staphylococcus aureus*, *Bacillus licheniformis* and *Streptomyces griseus*.
Lit. K. Breddam and M. Meldal, Eur. J. Biochem. ***206****, 103 (1992)*
Solubility: at least 1 mg/ml in 5 % sodium hydrogen carbonate with agitation

M-2480 **Abz-Gly-Ala-Ala-Pro-Phe-3-nitro-Tyr-Asp-OH**
Trifluoroacetate salt
Fluorometric substrate for Pro-specific endopeptidases.
Lit. E. Szwajcer-Dey et al., J. Bacteriol. ***174****, 2454 (1992)*
Solubility: at least 1mg/ml in methanol with agitation

H-2638 **Abz-Lys-Pro-Leu-Gly-Leu-Dap(Dnp)-Ala-Arg-NH$_2$**
Trifluoroacetate salt
Solubility: 10 mg/ml in water

N-Me-Abz/Dnp Substrates

Prod.No. Product

MMP Substrates

M-1910 **Dnp-Pro-β-cyclohexyl-Ala-Abu-Cys(Me)-His-Ala-Lys(N-Me-Abz)-NH_2**
An improved collagenase substrate with a better kcat/Km ratio than substrate M-1855. The Dnp group and the C-terminal N-methyl-anthranilyl moiety are fluorescence self-quenching until peptide cleavage occurs.
Lit. D.M. Bickett et al., Anal. Biochem. ***212****, 58 (1993)*
Solubility: in 50 % acetic acid

M-2055 **Dnp-Pro-β-cyclohexyl-Ala-Gly-Cys(Me)-His-Ala-Lys(N-Me-Abz)-NH_2**
Efficient fluorogenic substrate for two matrix metalloproteinases: interstitial collagenase (MMP-1) and gelatinase (MMP-9). This peptide has favorable solubility characteristics. Both enzymes cleave this substrate between Gly and Cys(Me), liberating a cleavage product with a fluorescence signal suitable for inhibitor screening and determining Ki values. The major advantage of this substrate is its adaptability to filters commonly available on commercial plate readers (excitation at 365 nm and emission at 450 nm).
Lit. D.M. Bickett et al., Anal. Biochem. ***212****, 58 (1993)/ L.J. Gould et al., In Vitro Cell. Dev. Biol. Anim.* ***35****, 75 (1999)/ J. Martin et al., J. Biol. Chem.* ***277****, 33683 (2002)*
Solubility: 10 mg/ml in 50 % acetic acid

Prod.No. Product

γ-Secretase Substrates

M-2555 **N-Me-Abz-Amyloid β/A4 Protein Precursor$_{770}$ (708-715)-Lys(Dnp)-D-Arg-D-Arg-D-Arg amide**
N-Me-Abz-Gly-Gly-Val-Val-Ile-Ala-Thr-Val-Lys(Dnp)-D-Arg-D-Arg-D-Arg-NH_2
(N-Me-Abz-APP$_{770}$ (708-715)-Lys(Dnp)-D-Arg-D-Arg-D-Arg amide; N-Me-Abz-Amyloid β-Protein (37-44)-Lys(Dnp)-D-Arg-D-Arg-D-Arg amide)
Trifluoroacetate salt
Novel intramolecularly quenched fluorescent, presenilin-dependent substrate for assaying γ-secretase activity. It has been used for partial purification and characterization of γ-secretase from post-mortem human brain.
Lit. M.R. Farmery et al., J. Biol. Chem. ***278****, 24277 (2003)*
Solubility: at least 1 mg/ml in water with agitation

Miscellaneous Substrates

M-2145 **N-Me-Abz-Lys-Pro-Leu-Gly-Leu-Dap(Dnp)-Ala-Arg-NH_2**
Trifluoroacetate salt
Solubility: 10 mg/ml in water

Dansyl/4-nitro-Phe Substrates

Prod.No. Product

Neprilysin Substrates

M-2650 **Dansyl-D-Ala-Gly-4-nitro-Phe-Glu-OH**
Trifluoroacetate salt
Highly sensitive fluorescence resonance energy transfer (FRET) substrate for neutral endopeptidase (NEP or neprilysin EC 3.4.24.11) (Km = 45 µM, kcat = 59 min^{-1}, kcat/Km = 1.3 $min^{-1}µM^{-1}$). Enzymatic activity can be monitored with an excitation wavelength of 342 nm and an emission wavelength of 562 nm.
Lit. D. Florentin et al., Anal. Biochem. ***141****, 62 (1984)/ V.H.J. van der Velden et al., Cytokine* ***10****, 55 (1998)/ C. Schmid et al., Regul. Peptides* ***130****, 57 (2005)*
Solubility: in 0.1 % TFA

Prod.No. Product

ADAM Protein Substrates

M-2545 **Glu(EDANS)-ADAM 8 (165-172)-Lys(DABCYL) amide (human)**
H-Glu(EDANS)-Arg-Thr-Ala-Ala-Val-Phe-Arg-Pro-Lys(DABCYL)-NH_2
(Glu(EDANS)-CD156a Antigen (165-172)-Lys(DABCYL) amide (human); Glu(EDANS)-Cell Surface Antigen MS2 (165-172)-Lys(DABCYL) amide (human); Glu(EDANS)-CD156 (165-172)-Lys(DABCYL) amide (human))
Trifluoroacetate salt
Novel fluorescent peptide substrate for ADAM 28 which is a member of the ADAM family of disintegrin metalloproteases.
Lit. A.M. Fourie et al., J. Biol. Chem. ***278****, 30469 (2003)*
Solubility: at least 1 mg/ml in DMSO or 20 % AcOH with agitation

M-2535 **H-Glu(EDANS)-Lys-Pro-Ala-Lys-Phe-Phe-Arg-Leu-Lys(DABCYL)-NH_2**
Trifluoroacetate salt
Novel quenched fluorescent substrate for ADAM 8, ADAM 15, and MDC-L (ADAM 28) but not for ADAM 17. For ADAM 8 half maximal cleavage was observed at 2.5 µM.
Lit. A.M. Fourie et al., J. Biol. Chem. ***278****, 30469 (2003)*
Solubility: at least 1 mg/ml in DMSO or 20 % acetic acid with agitation

Calpain-1 Substrates

M-2655 **H-Glu(EDANS)-Pro-Leu-Phe-Ala-Glu-Arg-Lys(DABCYL)-OH**
Internally quenched substrate for calpain-1 (µ-calpain) with optimal cleavage motifs flanking the scissile bond. The enzyme showed a more than 18-fold higher turnover rate for the hydrolysis of this FRET substrate based on the amino acid sequence PLFAER than for EVYGMM a sequence derived from the cleavage site of the natural substrate α-spectrin.
Lit. D. Cuerrier et al., J. Biol. Chem. ***280****, 40632 (2005)*
Solubility: at least 1 mg/ml in DMSO or 50 % acetic acid

Caspase-1 Substrates

M-1940 **DABCYL-Tyr-Val-Ala-Asp-Ala-Pro-Val-EDANS**
(DABCYL-YVADAPV-EDANS)
Trifluoroacetate salt
This fluorogenic caspase-1 (ICE) substrate is based on the principle of resonance energy transfer. It allows a continuous assay of caspase-1 that is useful in the screening of inhibitory compounds (Km = 11.4 µM, kcat = 0.79 s^{-1}).
Lit. M.W. Pennington and N.A. Thornberry, Peptide Res. ***7****, 72 (1994)/ M. Los et al., Nature* ***375****, 81 (1995)*
Solubility: at least 1 mg/ml in 50 % acetic acid

Prod.No. Product

Cathepsin Substrates

M-2295 **Ac-Glu-Asp(EDANS)-Lys-Pro-Ile-Leu-Phe-Phe-Arg-Leu-Gly-Lys(DABCYL)-Glu-NH_2**
Trifluoroacetate salt
Sensitive fluorescent peptide substrate for cathepsin D, an enzyme, that can degrade extracellular matrix components and may facilitate the spread of tumor cells. High levels of active cathepsin D were found within senile plaques in brains of Alzheimer's patients.
Lit. S.V. Gulnik et al., FEBS Lett. ***413****, 379 (1997)*
Solubility: at least 1 mg/ml in DMSO or 50 % acetic acid with agitation

Cytomegalovirus (CMV) Protease Substrates

M-2060 **Fluorogenic Human CMV Protease Substrate**
DABCYL-Arg-Gly-Val-Val-Asn-Ala-Ser-Ser-Arg-Leu-Ala-EDANS
Trifluoroacetate salt
This substrate has been synthesized to develop a fluorescence-based assay of human cytomegalovirus proteinase. It is cleaved specifically at the Ala-Ser bond thereby liberating the C-terminal peptide-EDANS fragment from the proximity quenching effect of the DABCYL group. This represents the first fluorescence-based assay of the herpes virus proteases and permits the characterization of potential inhibitors.
Lit. B.P. Holskin et al., Anal. Biochem. ***227****, 148 (1995)/ R. Batra et al., Nature Struct. Biol.* ***8****, 810 (2001)*
Solubility: at least 1 mg/ml in 20 % acetic acid with agitation

HCV NS3 Protease Substrates

M-2235 **Ac-Asp-Glu-Asp(EDANS)-Glu-Glu-Abu-L-lactoyl-Ser-Lys(DABCYL)-NH_2**
Trifluoroacetate salt
Internally quenched fluorogenic substrate for the continuous monitoring of HCV NS3 protease activity.
Lit. M. Taliani et al., Anal. Biochem. ***240****, 60 (1996)*
Solubility: at least 1 mg/ml in DMSO or 50 % acetic acid with agitation

Prod.No. Product

Hepatitis A Virus (HAV) 3C Protease Substrates

M-1900 **Hepatitis A Virus (HAV) 3C Protease Substrate**
DABCYL-Gly-Leu-Arg-Thr-Gln-Ser-Phe-Ser-EDANS
Trifluoroacetate salt
This fluorogenic substrate represents a cleavage sequence specific for the picornavirus 3C protease. Thus, it has been found to be highly sensitive for the 3C protease from hepatitis A virus (HAV).
Lit. M.W. Pennington et al., Peptides 1992, Proceedings of the 22nd European Peptide Symposium, Interlaken, Switzerland, p. 936, C.H. Schneider and A.N. Eberle, eds., Escom, Leiden (1993)
Solubility: at least 1 mg/ml in 50 % acetic acid with agitation

HIV Protease Substrates

M-1865 **DABCYL-γ-Abu-Ser-Gln-Asn-Tyr-Pro-Ile-Val-Gln-EDANS**
Trifluoroacetate salt
This fluorogenic HIV-1 protease substrate consists of an octapeptide with a fluorescent donor (EDANS) and a quenching acceptor (DABCYL), attached at the COOH- and NH_2-termini. The γ-Abu spacer was inserted to avoid potential steric hindrance of substrate binding by the bulky acceptor. This substrate is cleaved by the HIV-1 protease at the Tyr-Pro bond which results in a time-dependent increase in fluorescence intensity.
*Lit. E.D. Matayoshi et al., Science **247**, 954 (1990)/ M.W. Pennington et al., Peptides 1992, Proceedings of the 22nd European Peptide Symposium, Interlaken, Switzerland, p. 936, C.H. Schneider and A.N. Eberle, eds., Escom, Leiden (1993)*
Solubility: at least 1 mg/ml in DMSO or 80 % acetic acid with agitation

Kaposi's Sarcoma-Associated Herpes Virus (KSHV) Protease Substrates

M-2355 **H-Glu(EDANS)-Val-Tyr-Leu-Lys-Ala-Ser-Gln-Phe-Pro-Ala-Gly-Ile-Lys(DABCYL)-Gly-OH**
Trifluoroacetate salt
KSHV release site (R-site) substrate with a kcat/Km = $0.52 \cdot 10^3$ $M^{-1}min^{-1}$.
*Lit. T.R. Pray et al., J. Mol. Biol. **289**, 197 (1999)*
Solubility: at least 1 mg/ml in 20 % acetic acid with agitation

Prod.No. Product

Malaria Aspartyl Proteinase Substrates

M-2120 **DABCYL-Glu-Arg-Nle-Phe-Leu-Ser-Phe-Pro-EDANS**
Trifluoroacetate salt
Useful peptide substrate for a continuous fluorescence-based assay of the malaria aspartyl proteinase. The peptide sequence is derived from the cleavage site present in hemoglobin, with Nle as a substitution for Met to avoid potential oxidation related problems.
*Lit. M. Pennington et al., Innovation and Perspectives in Solid Phase Synthesis & Combinatorial Libraries, 5th International Symposium, London, p. 367, R. Epton, ed., Mayflower Scientific (1998)/ S. Jiang et al., Antimicrob. Agents Chemother. **45**, 2577 (2001)*
Solubility: at least 1 mg/ml in DMSO or 50 % acetic acid with agitation

MMP Substrates

M-2490 **DABCYL-γ-Abu-Arg-Pro-Lys-Pro-Val-Glu-Nva-Trp-Arg-Glu(EDANS)-Ala-Lys-NH_2**
A selective fluorogenic matrix metalloproteinase (MMP) substrate. The kcat/Km value for MMP-3 (Stromelysin-1) is 29000 $M^{-1}s^{-1}$, for MMP-13 13000 $M^{-1}s^{-1}$, and for MMP-9 7500 $M^{-1}s^{-1}$ (at pH 7.5, 25 °C). The fluorescence increase is monitored using an excitation wavelength of 360 nm and an emission wavelength of 490 nm. No activity against this substrate could be measured with MMP1, MMP-7, and MMP-8 (kcat/Km < 1000 $M^{-1}s^{-1}$).
*Lit. B. Beekman et al., FEBS Lett. **418**, 305 (1997)*
Solubility: in water

M-2495 **DABCYL-γ-Abu-Pro-Gln-Gly-Leu-Glu(EDANS)-Ala-Lys-NH_2**
Highly soluble fluorogenic matrix metalloproteinase (MMP) substrate with the following kcat/Km values (at pH 7.6, 37 °C): 619000 $M^{-1}s^{-1}$ for MMP-2, 209000 $M^{-1}s^{-1}$ for MMP-9, 40000 $M^{-1}s^{-1}$ for MMP-3, and 21000 $M^{-1}s^{-1}$ for MMP-1. The fluorescence increase is measured using an excitation wavelength of 340 nm and an emission wavelength of 485 nm. Due to the relatively high emission wavelength of EDANS the substrate allows convenient measurement of MMP activity in complex biological media like synovial fluid and culture medium.
*Lit. B. Beekman et al., FEBS Lett. **390**, 221 (1996)*
Solubility: 1 mg/ml in water

Prod.No. Product

Renin Substrates

M-2050 **DABCYL-γ-Abu-Ile-His-Pro-Phe-His-Leu-Val-Ile-His-Thr-EDANS**
Trifluoroacetate salt
This fluorogenic peptide substrate has been developed to continuously measure the proteolytic activity of human renin (Km = 1.5 µM at physiological pH). Cleavage of the substrate occurs specifically at the Leu-Val bond and corresponds to the renin cleavage site of angiotensinogen. By means of this assay as low as 30 ng/ml renin can be detected after an incubation of only 3-5 min.
Lit. G.T. Wang et al., Anal. Biochem. ***210****, 351 (1993)*
Solubility: at least 1 mg/ml in DMSO or 50 % acetic acid with agitation

SARS Main Protease Substrates

M-2575 **DABCYL-Lys-HCoV-SARS Replicase Polyprotein 1ab (3235-3246)-Glu-EDANS**
DABCYL-Lys-Thr-Ser-Ala-Val-Leu-Gln-Ser-Gly-Phe-Arg-Lys-Met-Glu-EDANS
(DABCYL-Lys-pp1ab (3235-3246)-Glu-EDANS (Human coronavirus) (strain SARS); DABCYL-Lys-ORF1AB (3235-3246)-Glu-EDANS (Human coronavirus) (strain SARS))
Trifluoroacetate salt
Sensitive internally quenched fluorogenic substrate for SARS main protease with a Km value of 17 µM and a kcat value of 1.9 s^{-1}.
Lit. C.-J. Kuo et al., Biochem. Biophys. Res. Commun. ***318****, 862 (2004)*
Solubility: 1 mg/ml in 50 % acetic acid

β-Secretase Substrates

M-2445 **DABCYL-(Asn^{670},Leu^{671})-Amyloid β/A4 Protein Precursor$_{770}$ (661-675)-EDANS**
DABCYL-Ile-Lys-Thr-Glu-Glu-Ile-Ser-Glu-Val-Asn-Leu-Asp-Ala-Glu-Phe-EDANS
(DABCYL-(Asn^{670},Leu^{671})-APP$_{770}$ (661-675)-EDANS)
Ammonium salt
Solubility: 1 mg/ml in TFA

M-2435 **DABCYL-(Asn^{670},Leu^{671})-Amyloid β/A4 Protein Precursor$_{770}$ (667-675)-EDANS**
DABCYL-Ser-Glu-Val-Asn-Leu-Asp-Ala-Glu-Phe-EDANS
(DABCYL-(Asn^{670},Leu^{671})-APP$_{770}$ (667-675)-EDANS)
Ammonium salt
Solubility: 1 mg/ml in water

Prod.No. Product

M-2470 **Arg-Glu(EDANS)-(Asn^{670},Leu^{671})-Amyloid β/A4 Protein Precursor$_{770}$ (668-675)-Lys(DABCYL)-Arg**
H-Arg-Glu(EDANS)-Glu-Val-Asn-Leu-Asp-Ala-Glu-Phe-Lys(DABCYL)-Arg-OH
(Arg-Glu(EDANS)-(Asn^{670},Leu^{671})-APP$_{770}$ (668-675)-Lys(DABCYL)-Arg)
Trifluoroacetate salt
Fluorogenic substrate for pro-memapsin-2 containing the β-secretase site of the Swedish mutation of APP. The kinetic parameters at pH 4.5 are Km = 5.4 µM and kcat = 0.24 min^{-1}.
Lit. A.K. Ghosh et al., J. Am. Chem. Soc. ***122****, 3522 (2000)/ J. Ermolieff et al., Biochemistry* ***39****, 12450 (2000)*
Solubility: 1 mg/ml in DMSO

M-2430 **DABCYL-(Asn^{670},Leu^{671})-Amyloid β/A4 Protein Precursor$_{770}$ (669-674)-EDANS**
DABCYL-Val-Asn-Leu-Asp-Ala-Glu-EDANS
(DABCYL-(Asn^{670},Leu^{671})-APP$_{770}$ (669-674)-EDANS)
Ammonium salt
Solubility: 1 mg/ml in water

TNF-α Converting Enzyme Substrates

M-2155 **DABCYL-TNF-α-EDANS (-4 to +6) (human)**
DABCYL-Leu-Ala-Gln-Ala-Val-Arg-Ser-Ser-Ser-Arg-EDANS
Trifluoroacetate salt
This substrate represents the optimal size for efficient fluorescent quenching, while incorporating all of the necessary amino acids to yield a viable substrate to be used for rapid screening of compounds for inhibition of TNF-α converting enzyme (TACE) activity.
Lit. A.J.H. Gearing et al., Nature ***370****, 555 (1994)/ K.M. Mohler et al., Nature* ***370****, 218 (1994)/ B.F. Becker et al., Biol. Chem.* ***383****, 1821 (2002)*
Solubility: at least 1 mg/ml in 50 % acetic acid with agitation

Miscellaneous Substrates

M-2380 **DABCYL-(Nle^{1077})-Collagen Type III α1 chain (1070-1077)-EDANS (human)**
DABCYL-Pro-Tyr-Tyr-Gly-Asp-Glu-Pro-Nle-EDANS
(DABCYL-(Asp^{1067},Nle^{1069})-Collagen Type III α1 chain (1062-1069)-EDANS (mouse))
Substrate for metalloproteinases.
Solubility: 1 mg/ml in methanol

FITC/Dnp Substrates

Prod.No. **Product**

Caspase-1 Substrates

M-2285 **FITC-Tyr-Val-Ala-Asp-Ala-Pro-Lys(Dnp)-OH (Contains FITC isomer I)**
(FITC-YVADAPK(Dnp))
Specific substrate for the determination of caspase-1 and caspase-1 like enzyme activities. Cleavage of this peptide substrate at the P1 Asp residue results in a continuous fluorescent assay. It is useful both in FACS and fluorescence microscopy experiments. Caspase-3 is only weakly active using this substrate. See also our reference substance M-2280.
*Lit. H.J. Korting et al., Virologie **28**, 41 (1977)/ A. Chersi et al., Biochim. Biophys. Acta **1034**, 333 (1990)*
Solubility: in basic solvents

Lucifer Yellow/Dabsyl Substrates

Prod.No. **Product**

β-Secretase Substrates

M-2570 **Lys(Dabsyl)-(Asn^{670},Leu^{671})-Amyloid β/A4 Protein Precursor$_{770}$ (667-676)-Gln-Lucifer Yellow**
H-Lys(Dabsyl)-Ser-Glu-Val-Asn-Leu-Asp-Ala-Glu-Phe-Arg-Gln-Lucifer Yellow
(Lys(Dabsyl)-(Asn^{670},Leu^{671})-APP$_{770}$ (667-676)-Gln-Lucifer Yellow)
Ammonium salt
A highly selective substrate for measuring BACE1 (Km = 9 µM, kcat = 0.02 s^{-1}) and BACE2 activity. In this fluorescence resonance energy transfer (FRET) substrate the fluorescent group Lucifer Yellow is efficiently quenched by Dabsyl (4-(4-Dimethylaminophenylazo)benzenesulfonyl). Enzymatic cleavage can be assayed by detecting the increase in fluorescence emission at 520 nm using an excitation wavelength of 430 nm.
*Lit. F. Grüninger-Leitch et al., J. Biol. Chem. **277**, 4687 (2002)*
Solubility: 1 mg/ml in 1 N ammonia

Prod.No. Product

Caspase-1 Substrates

M-2195 **Mca-Tyr-Val-Ala-Asp-Ala-Pro-Lys(Dnp)-OH**
(Mca-YVADAPK(Dnp))
Trifluoroacetate salt
Specific highly fluorescent substrate for the determination of caspase-1 (ICE) and caspase-1-like enzyme activities. Cleavage of this peptide substrate at the P1 Asp residue results in a continuous fluorescent assay monitored at an emission wavelength of 392 nm. Caspase-3 (apopain, CPP-32) is only weakly active on this substrate. Both caspase-1 and caspase-3 are involved in apoptosis.
Lit. M. Enari et al., Nature ***380****, 723 (1996)*
Solubility: at least 1 mg/ml in 80 % acetic acid or DMSO with agitation

Caspase-3 Substrates

M-2200 **Mca-Asp-Glu-Val-Asp-Ala-Pro-Lys(Dnp)-OH**
(Mca-DEVDAPK(Dnp))
Trifluoroacetate salt
Specific highly fluorescent substrate for the determination of caspase-3 (also named apopain or CPP-32) and CPP-32-like enzyme activities. Cleavage of this peptide substrate at the P1 Asp residue results in a continuous fluorescent assay monitored at an emission wavelength of 392 nm. Caspase-1 has only little activity on this substrate.
Lit. M. Enari et al., Nature ***380****, 723 (1996)*
Solubility: at least 1 mg/ml in 0.1 M sodium hydrogen carbonate with agitation

Caspase-4 Substrates

M-2315 **Mca-Leu-Glu-Val-Asp-Gly-Trp-Lys(Dnp)-NH_2**
(Mca-LEVDGWK(Dnp)-NH_2)
This fluorogenic resonance energy transfer substrate for caspase-4 (ICH-2) exhibits an excitation wavelength of 325 nm and an emission wavelength of 392 nm.
Lit. R.V. Talanian et al., J. Biol. Chem. ***272****, 9677 (1997)*
Solubility: in TFA and DMSO

Prod.No. Product

Cathepsin Substrates

M-2455 **Mca-Gly-Lys-Pro-Ile-Leu-Phe-Phe-Arg-Leu-Lys(Dnp)-D-Arg-NH_2**
Trifluoroacetate salt
Substrate for cathepsin D and E.
Lit. Y. Yasuda et al., J. Biochem. ***125****, 1137 (1999)*
Solubility: 1 mg/ml in 50 % acetic acid

M-2625 **Mca-Gly-Ser-Pro-Ala-Phe-Leu-Ala-Lys(Dnp)-D-Arg-NH_2**
Trifluoroacetate salt
Highly sensitive and selective cathepsin E FRET substrate derived from the cleavage site sequence of human α2-macroglobulin (Km = 1.9 µM; kcat/Km = 10.2 $\mu M^{-1}s^{-1}$ for human erythrocyte cathepsin E). The substrate was resistent to hydrolysis by the analogous aspartic proteinases cathepsin D and pepsin, as well as the lysosomal cysteine proteinases cathepsin B, L, and H. Useful for monitoring and accurately quantifying cathepsin E, even in crude enzyme preparations (excitation wavelength at 328 nm, emission wavelength at 393 nm).
Lit. Y. Yasuda et al., Biol. Chem. ***386****, 299 (2005)*
Solubility: in 0.1 % TFA in acetonitrile/water

Endothelin-Converting Enzyme-1 Substrates

M-2405 **Mca-(Ala^7,Lys(Dnp)^9)-Bradykinin**
Mca-Arg-Pro-Pro-Gly-Phe-Ser-Ala-Phe-Lys(Dnp)-OH
Trifluoroacetate salt
Very sensitive internally quenched fluorescent substrate for endothelin-converting enzyme-1 (ECE-1) a membrane-bound zinc metallopeptidase that is related to neprilysin in amino acid sequence. The kcat/Km value for the hydrolysis by ECE-1 was $1.9 \cdot 10^7$ $M^{-1}s^{-1}$ and $1.7 \cdot 10^6$ $M^{-1}s^{-1}$ for the hydrolysis by neprilysin. For MMP-2 and MMP-9 the kcat/Km values are in the range of 10^4 $M^{-1}s^{-1}$ whereas for MMP-1 there was no hydrolysis observed.
Lit. G.D. Johnson and K. Ahn, Anal. Biochem. **286***, 112 (2000)*
Solubility: 1 mg/ml in 80 % acetic acid

Human Herpes Virus 8 (HHV-8) Protease Substrates

M-2260 **Mca-γ-Abu-Asn-Arg-Leu-Glu-Ala-Ser-Ser-Arg-Ser-Ser-Lys(Dnp)-NH_2**
Trifluoroacetate salt
Fluorogenic substrate containing the M-site of Kaposi's sarcoma-associated herpes virus (KSHV) (also called human herpes virus 8 (HHV-8)) for the assay of KSHV protease (kcat/Km = 165 $M^{-1}s^{-1}$). Time-evolved fluorescence enhancement was monitored by exciting this KSHV M-site peptide at 325 nm and detecting emission at 393 nm.
Lit. A. Uenal et al., J. Virol. ***71****, 7030 (1997)*
Solubility: at least 1 mg/ml in 80 % acetic acid with agitation

Prod.No. Product

MMP Substrates

M-2105 **Mca-Arg-Pro-Lys-Pro-Tyr-Ala-Nva-Trp-Met-Lys(Dnp)-NH_2**
Trifluoroacetate salt
This substrate was hydrolyzed 60 times more rapidly by stromelysin 1 (MMP-3) (kcat/Km = 59400 $M^{-1}s^{-1}$) than by interstitial collagenase (MMP-1). However it showed little discrimination between MMP-3, gelatinase A (MMP-2) (kcat/Km = 54000 $M^{-1}s^{-1}$), and gelatinase B (MMP-9) (kcat/Km = 55300 $M^{-1}s^{-1}$).
Lit. H. Nagase et al., J. Biol. Chem. ***269****, 20952 (1994)*
Solubility: at least 1 mg/ml in 20 % acetic acid

M-2110 **Mca-Arg-Pro-Lys-Pro-Val-Glu-Nva-Trp-Arg-Lys(Dnp)-NH_2**
Trifluoroacetate salt
Was hydrolyzed rapidly by stromelysin 1 (MMP-3) with kcat/Km = 218000 $M^{-1}s^{-1}$ and very slowly by gelatinase B (MMP-9) (kcat/Km = 10100 $M^{-1}s^{-1}$). There was no hydrolysis by MMP-1 and MMP-2.
Lit. H. Nagase et al., J. Biol. Chem. ***269****, 20952 (1994)*
Solubility: at least 1 mg/ml in water with agitation

M-2350 **Mca-Lys-Pro-Leu-Gly-Leu-Dap(Dnp)-Ala-Arg-NH_2**
Trifluoroacetate salt
The N-terminal elongation of the widely used MMP substrate Mca-Pro-Leu-Gly-Leu-Dap(Dnp)-Ala-Arg-NH_2 (M-1895) with a Lys yields a fluorogenic peptide with improved substrate properties. As compared to M-1895, the specificity constant (kcat/Km) of M-2350 for collagenases (MMP-1, MMP-8, MMP-13) and MMP-14 is increased two- to ninefold and threefold, respectively, while those for gelatinases and matrilysin remain equally high.
Lit. U. Neumann et al., Anal. Biochem. ***328****, 166 (2004)*
Solubility: 5 mg/ml in 50 % acetic acid

M-2515 **Mca-Pro-β-cyclohexyl-Ala-Gly-Nva-His-Ala-Dap(Dnp)-NH_2**
Trifluoroacetate salt
Specific fluorogenic substrate for collagenase-3 (MMP-13) with a kcat/Km value of $1.09 \cdot 10^6$ $M^{-1}s^{-1}$ (pH = 7.5, T = 25 °C, substrate concentrations: 0.7 and 1.4 µM (S << Km), determined for activated recombinant collagenase-3). Collagenase-3 hydrolyzed this substrate 70 - 100 fold more efficiently than collagenase-2 (MMP-8) and collagenase-1 (MMP-1).
Lit. V. Knäuper et al., J. Biol. Chem. ***271****, 1544 (1996)/ L. Howard et al., FEBS Lett.* ***498****, 82 (2001)*
Solubility: 2 mg/ml in 50 % acetic acid

M-2510 **Mca-Pro-Leu-Ala-Cys(Mob)-Trp-Ala-Arg-Dap(Dnp)-NH_2**
Trifluoroacetate salt
Fluorogenic substrate for MMP-14 (MT1-MMP) and stromelysin-3 (ST3). It displayed a kcat/Km value of $3.6 \cdot 10^4$ $M^{-1}s^{-1}$ and $7.3 \cdot 10^5$ $M^{-1}s^{-1}$, when assayed with ST3 and MMP-14, respectively.
Lit. A. Mucha et al., J. Biol. Chem. ***273****, 2763 (1998)*
Solubility: 5 mg/ml in 50 % acetic acid

Prod.No. Product

M-2520 **Mca-Pro-Leu-Ala-Nva-Dap(Dnp)-Ala-Arg-NH_2**
Trifluoroacetate salt
Among the fluorescent peptide substrates analyzed, Mca-Pro-Leu-Ala-Nva-Dap(Dnp)-Ala-Arg-NH_2 displayed the highest specificity constant with MMP-26 (kcat/Km = $3.0 \cdot 10^4$ $M^{-1}s^{-1}$, pH 7.5, T = 25 °C) . The fluorescence was measured at an excitation wavelength of 328 nm and emission wavelength of 393 nm.
Lit. L. Howard et al., FEBS Lett. ***498****, 82 (2001)/ V. Knäuper et al., Eur. J. Biochem.* ***268****, 1888 (2001)/ H.I. Park et al., J. Biol. Chem.* ***277****, 35168 (2002)*
Solubility: in TFA buffer

M-1895 **Mca-Pro-Leu-Gly-Leu-Dap(Dnp)-Ala-Arg-NH_2**
Trifluoroacetate salt
This fluorogenic peptide is a very sensitive substrate for continuous assays and *in situ* determination of the matrix metalloproteinase activity. Cleavage at the Gly-Leu bond separates the highly fluorescent Mca group from the efficient 2,4-dinitrophenyl quencher, resulting in an increase in fluorescence intensity. The kcat/Km values for the punctuated metalloproteinase (MMP-7) and for gelatinase (MMP-2), e.g., are $1.7 \cdot 10^5$ and $6.3 \cdot 10^5$ $M^{-1}s^{-1}$, respectively.
Lit. C.G. Knight et al., FEBS Lett. ***296****, 263 (1992)/ Z.S. Galis et al., FASEB J.* ***9****, 974 (1995)/ M.G. Natchus et al., J. Med. Chem.* ***43****, 4948 (2000)/ U. Neumann et al., Anal. Biochem.* ***328****, 166 (2004)*
Solubility: 10 mg/ml in 50 % acetic acid

M-2670 **Mca-Pro-Leu-Gly-Leu-Glu-Glu-Ala-Dap(Dnp)-NH_2**
Trifluoroacetate salt
Highly selective MMP-12 FRET substrate with a kcat/Km value of $1.85 \cdot 10^5$ $M^{-1}s^{-1}$. Poor substrate of other MMPs with the exception of MMP-13 (kcat/Km = $0.53 \cdot 10^5$ $M^{-1}s^{-1}$) and MMP-9 ($0.33 \cdot 10^5$ $M^{-1}s^{-1}$).
Lit. L. Devel et al., J. Biol. Chem. ***281****, 11152 (2006)*
Solubility: in 45 % acetonitrile in 0.1 % ammonia

β-Secretase Substrates

M-2425 **Mca-(Asn^{670},Leu^{671})-Amyloid β/A4 Protein Precursor$_{770}$ (667-674)-Dap(Dnp)**
Mca-Ser-Glu-Val-Asn-Leu-Asp-Ala-Glu-Dap(Dnp)-OH
(Mca-(Asn^{670},Leu^{671})-APP_{770} (667-674)-Dap(Dnp))
Ammonium acetate salt
Solubility: 1 mg/ml in TFA or 0.1 mg/ml in water

M-2420 **Mca-(Asn^{670},Leu^{671})-Amyloid β/A4 Protein Precursor$_{770}$ (667-675)-Lys(Dnp)**
Mca-Ser-Glu-Val-Asn-Leu-Asp-Ala-Glu-Phe-Lys(Dnp)-OH
(Mca-(Asn^{670},Leu^{671})-APP_{770} (667-675)-Lys(Dnp))
Ammonium acetate salt
Fluorogenic substrate for pro-memapsin-2 containing the β-secretase site of the Swedish mutation of APP.
Solubility: 1 mg/ml in 0.5 % ammonium hydroxide

Prod.No. Product

M-2485 **Mca-(Asn^{670},Leu^{671})-Amyloid β/A4 Protein Precursor$_{770}$ (667-675)-Lys(Dnp) amide**
Mca-Ser-Glu-Val-Asn-Leu-Asp-Ala-Glu-Phe-Lys(Dnp)-NH_2
(Mca-(Asn^{670},Leu^{671})-APP$_{770}$ (667-675)-Lys(Dnp) amide)
Trifluoroacetate salt
Fluorogenic substrate for pro-memapsin-2 containing the β-secretase site of the Swedish mutation of APP. The kinetic parameters at pH 4.5 are Km = 4.5 µM and kcat = 0.25 min^{-1}.
Lit. J. Ermolieff et al., Biochemistry ***39****, 16263 (2000)/ J. Ermolieff et al., Biochemistry* ***39****, 12450 (2000)*
Solubility: at least 1 mg/ml in DMSO with agitation

M-2460 **Mca-Amyloid β/A4 Protein Precursor$_{770}$ (667-676)-Lys(Dnp)-Arg-Arg amide**
Mca-Ser-Glu-Val-Lys-Met-Asp-Ala-Glu-Phe-Arg-Lys(Dnp)-Arg-Arg-NH_2
(Mca-APP$_{770}$ (667-676)-Lys(Dnp)-Arg-Arg amide)
Trifluoroacetate salt
This fluorescent peptide substrate contains the wild-type amyloid precursor protein (APP) β-secretase cleavage site. It has been used for assaying β-secretase-like activity of thimet oligopeptidase (TOP, EC 3.4.24.15). The results suggested that TOP is a potential β-secretase candidate and is involved in the processing of APP *in vivo*.
Lit. H. Koike et al., J. Biochem. ***126****, 235 (1999)*
Solubility: 1 mg/ml in water

M-2465 **Mca-(Asn^{670},Leu^{671})-Amyloid β/A4 Protein Precursor$_{770}$ (667-676)-Lys(Dnp)-Arg-Arg amide**
Mca-Ser-Glu-Val-Asn-Leu-Asp-Ala-Glu-Phe-Arg-Lys(Dnp)-Arg-Arg-NH_2
(Mca-(Asn^{670},Leu^{671})-APP$_{770}$ (667-676)-Lys(Dnp)-Arg-Arg amide)
Trifluoroacetate salt
This fluorescent peptide substrate contains the ‚Swedish' Lys-Met/Asn-Leu mutation of the amyloid precursor protein (APP) β-secretase cleavage site. It has been used for assaying β-secretase-like activity of thimet oligopeptidase (TOP, EC 3.4.24.15). The results suggested that TOP is a potential β-secretase candidate and is involved in the processing of APP *in vivo*.
Lit. H. Koike et al., J. Biochem. ***126****, 235 (1999)*
Solubility: 1 mg/ml in 50 % TFA

M-2440 **Mca-(Asn^{670},Leu^{671})-Amyloid β/A4 Protein Precursor$_{770}$ (669-674)-Lys(Dnp)**
Mca-Val-Asn-Leu-Asp-Ala-Glu-Lys(Dnp)-OH
(Mca-(Asn^{670},Leu^{671})-APP$_{770}$ (669-674)-Lys(Dnp))
Solubility: 10 mg/ml in 1 % ammonium hydroxide

Prod.No. Product

Thimet Oligopeptidase Substrates

M-2270 **Mca-Pro-Leu-Gly-Pro-D-Lys(Dnp)-OH**
Specific highly fluorescent substrate for the determination of the thimet oligopeptidase (EP 24.15, EC 3.4.24.15) activity.
Lit. A.J. Wolfson et al., Biochem. Biophys. Res. Commun. ***229****, 341 (1996)*
Solubility: 10 mg/ml in 50 % acetic acid

TNF-α Converting Enzyme Substrates

M-2255 **Mca-(endo-1a-Dap(Dnp))-TNF-α (-5 to +6) amide (human)**
Mca-Pro-Leu-Ala-Gln-Ala-Val-Dap(Dnp)-Arg-Ser-Ser-Ser-Arg-NH_2
Trifluoroacetate salt
Fluorogenic peptide substrate for tumor necrosis factor (TNF) converting enzyme.
Lit. D.E. Van Dyk et al., Bioorg. Med. Chem. Lett ***7****, 1219 (1997)*
Solubility: at least 1 mg/ml in ½ portion of acetonitrile, then ½ portion of water with agitation

Miscellaneous Substrates

M-2390 **Mca-Arg-Pro-Leu-Ala-Leu-Trp-Arg-Dap(Dnp)-NH_2**
Trifluoroacetate salt
Solubility: 1 mg/ml in 50 % acetic acid

M-2225 **Mca-Pro-Lys-Pro-Leu-Ala-Leu-Dap(Dnp)-Ala-Arg-NH_2**
Trifluoroacetate salt
Lit. G.N. Marchenko et al., Biochem. J. ***356****, 705 (2001)/ D.R. Hurst et al., Biochem. J.* ***377****, 775 (2004)*
Solubility: at least 1 mg/ml in 0.1 % TFA

M-2395 **Mca-(Gln^{192})-Succinate Semialdehyde Dehydrogenase (186-192)-Dap(Dnp) amide (human, E. coli)**
Mca-Thr-Pro-Phe-Ser-Ala-Leu-Gln-Dap(Dnp)-NH_2
Trifluoroacetate salt
Solubility: 1 mg/ml in 50 % acetic acid

Trp/Dnp Substrates

Prod.No. Product

MMP Substrates

M-2205 **Dnp-Arg-Pro-Leu-Ala-Leu-Trp-Arg-Ser-OH**
Trifluoroacetate salt
This peptide is the best fluorogenic substrate developed for matrilysin (also called punctuated metalloproteinase 1, PUMP-1 or MMP-7) thus far. In addition to good kinetic parameters (kcat/Km = $1.9 \cdot 10^5$ $M^{-1}s^{-1}$), it showed good solubility in assay buffer.
Lit. A.R. Welch et al., Arch. Biochem. Biophys. ***324****, 59 (1995)*
Solubility: at least 1 mg/ml in 10 % acetic acid with agitation

M-1855 **Dnp-Pro-Leu-Gly-Leu-Trp-Ala-D-Arg-NH_2**
Trifluoroacetate salt
This fluorogenic peptide is an efficient substrate for interstitial/vertebrate collagenase (MMP-1), stromelysin (MMP-3), and especially for the gelatinases (MMP-2 and MMP-9) and the punctuated metalloproteinase (MMP-7).
Lit. Y. Masui et al., Biochem. Med. ***17****, 215 (1977)/ M.S. Stack and R.D. Gray, J. Biol. Chem.* ***264****, 4277 (1989)/ K. Darlak et al., J. Biol. Chem.* ***265****, 5199 (1990)/ C.G. Knight et al., FEBS Lett.* ***296****, 263 (1992)/ D.M. Bickett et al., Anal. Biochem.* ***212****, 58 (1993)*
Solubility: at least 1 mg/ml in 50 % acetic acid with agitation

Trp/4-nitro-Z Substrates

Prod.No. Product

MMP Substrates

M-1595 **4-Nitro-Z-Gly-Trp-Gly-OH**
Fluorogenic substrate for a simple, sensitive, and reproducible assay of angiotensin I-converting enzyme, especially in human plasma.
Lit. A. Persson and I.B. Wilson, Anal. Biochem. ***83****, 296 (1977)/ A. Persson and I.B. Wilson, Anal. Biochem.* ***86****, 616 (1978)/ S.F. Russo et al., Clin. Chem.* ***24****, 1539 (1978)*
Solubility: 50 mg/ml in methanol

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