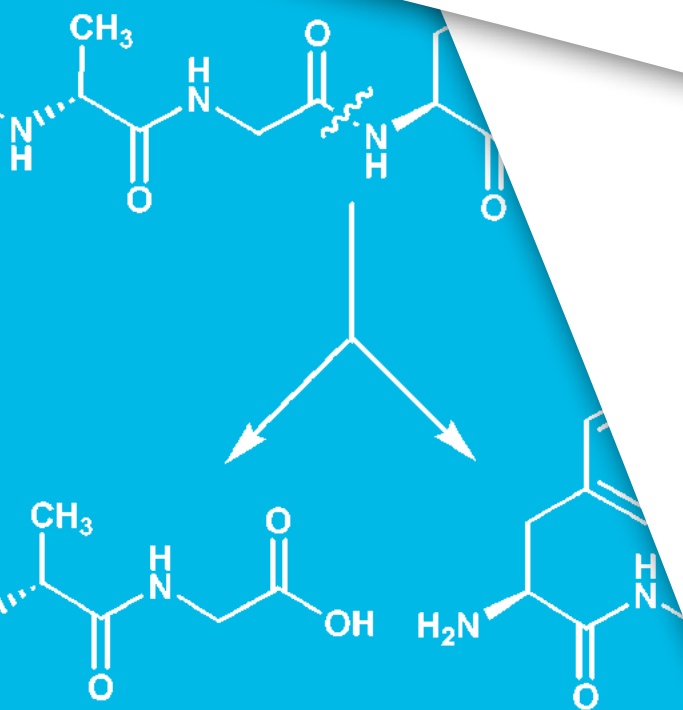


# FRET SUBSTRATES BACHEM

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# FRET SUBSTRATES OFFERED BY BACHEM

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. FRET is used in a variety of applications including the measurement of protease activity with substrates, in which the fluorophore is separated from the quencher by a short peptide sequence containing the enzyme cleavage site. Proteolysis of the peptide results in fluorescence as the fluorophore and quencher are separated. In this brochure we present 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 is illustrated by the Jablonski energy level diagram (Fig 1). 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.

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 maximum 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. The fluorescence spectra of fluorophores containing acidic or basic substituents (e.g. AMC) can depend on the pH of the solution.

### 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. 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.

### 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 (Fig 2). The energy transfer can be considered as an energy exchange of an oscillating dipole to a dipole with similar

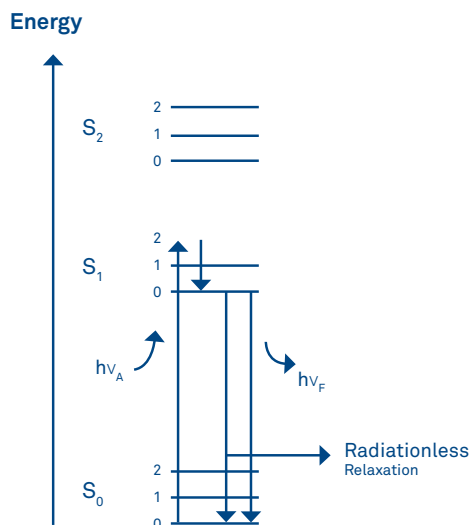


Fig. 1.  
Energy Level Diagram

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  $R_0$ .

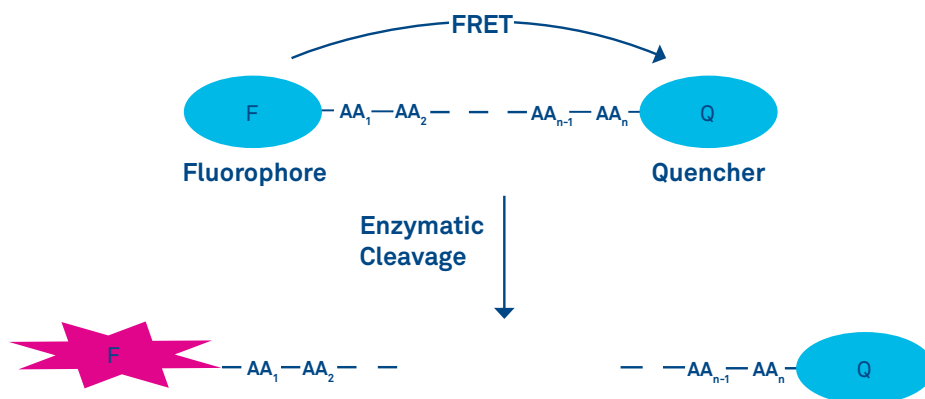
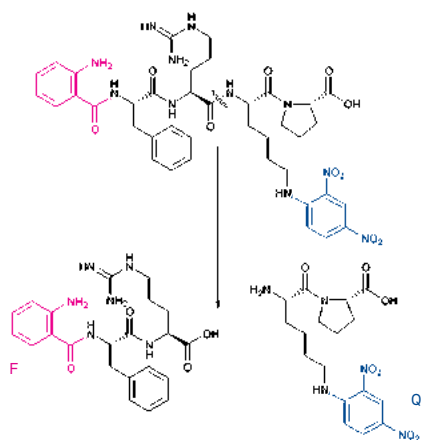


Fig. 2.  
Fluorescence Resonance Energy Transfer (FRET)

**Abz (2-Aminobenzoyl or Anthraniloyl) Substrates**

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

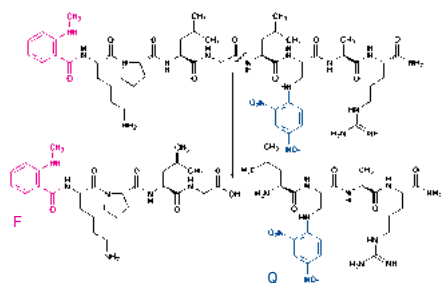
Example: 4043877 Abz-Phe-Arg-Lys(Dnp)-Pro-OH



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

N-Me-Abz substrates are generally used with Dnp as quencher (Q). The fluorescent group (F) is either linked to the N-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.

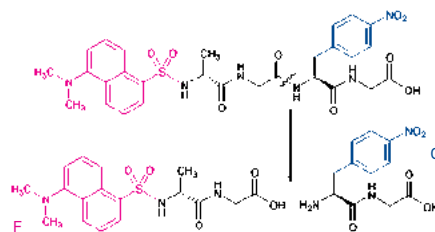
Example: N-Me-Abz-Lys-Pro-Leu-Gly-Leu-Dap(Dnp)-Ala-Arg-NH<sub>2</sub>



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

In a few substrates the fluorescent dansyl group (F) serves as donor with 4-nitro-phenylalanine as acceptor. 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.

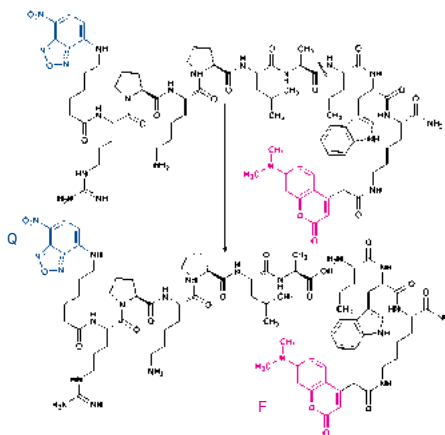
Example: 4050412 Dansyl-D-Ala-Gly-4-nitro-Phe-Gly-OH



**DMACA (7-Dimethylaminocoumarin-4-acetyl) Substrates**

DMACA (F) can be detected fluorometrically at 465 nm using an excitation wavelength of 350 nm. It can be quenched by NBD (7-Nitro-benzo[2,1,3]oxadiazol-4-yl) (Q).

Example: 4028275 NBD-ε-aminocaproyl-Arg-Pro-Lys-Pro-Leu-Ala-Nva-Trp-Lys(DMACA)-NH<sub>2</sub>

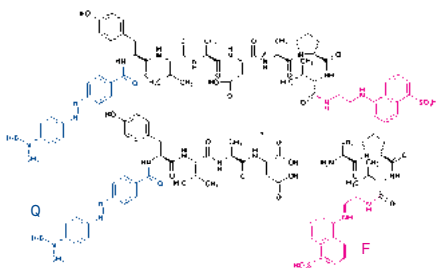


### EDANS (5-[(2-Aminoethyl)amino]naphthalene-1-sulfonic acid) Substrates

In these substrates, the fluorescence of the EDANS group (F) is generally quenched by the DABCYL (4-(4-dimethylaminophenylazo) benzoyl) group (Q). The DABCYL group is usually conjugated to the N-terminus and the EDANS group attached to the C-terminus of the peptide substrate.

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

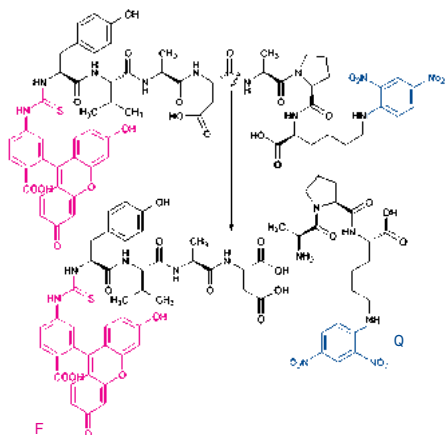
Example: DABCYL-Tyr-Val-Ala-Asp-Ala-Pro-Val-EDANS



### FITC (Fluorescein isothiocyanate) Substrates

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

Example: 4027937 FITC-Tyr-Val-Ala-Asp-Ala-Pro-Lys(Dnp)-OH (contains FITC isomer I)

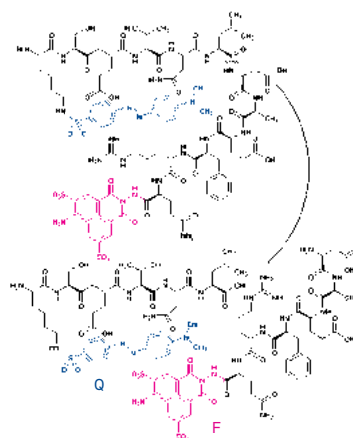


### Lucifer Yellow (6-Amino-2,3-dihydro-1,3-dioxo-2-hydrazinocarbonylamino-1H-benz[d,e]isoquinoline-5,8-disulfonic acid) Substrates

Lucifer Yellow (F) can be detected at 520 nm using excitation at 430 nm.

It is efficiently quenched by Dabsyl (4-(4-Dimethylaminophenylazo)-benzene-sulfonyl) (Q).

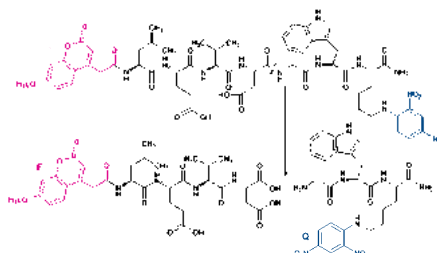
Example: H-Lys(Dabsyl)-Ser-Glu-Val-Asn-Leu-Asp-Ala-Glu-Phe-Arg-Gln-Lucifer Yellow



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

In this kind of substrates Mca (F) 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.

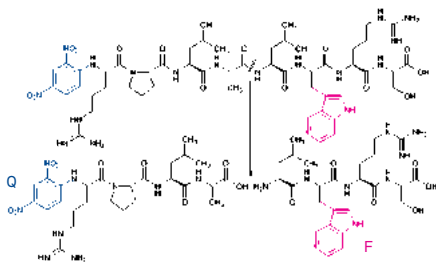
Example: Mca-Leu-Glu-Val-Asp-Gly-Trp-Lys(Dnp)-NH<sub>2</sub>



### Trp (Tryptophan) Substrates

Tryptophan (F) 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.

Example: 4030541 Dnp-Arg-Pro-Leu-Ala-Leu-Trp-Arg-Ser-OH



**Table 1.**  
Fluorophores

Fluorophore	Excitation Wavelength*	Emission Wavelength*	References
<b>Abz</b> (2-Aminobenzoyl or Anthraniloyl)	320 nm	420 nm	Cezari, M.H. et al. (2002); Bourgeois, L. et al. (1997); Parameswaran, K.N. et al. (1997)
<b>N-Me-Abz</b> (N-Methyl-anthraniloyl)	340-360 nm	440-450 nm	Bickett, D.M. et al. (1993)
<b>Dansyl</b> (5-(Dimethylamino)naphthalene-1-sulfonyl)	342 nm	562 nm	Florentin, D. et al. (1984)
<b>DMACA</b> (7-Dimethylaminocoumarin-4-acetate)	350 nm	465 nm	Bickett, D.M. et al. (1994)
<b>EDANS</b> (5-[(2-Aminoethyl)amino]naphthalene-1-sulfonic acid)	340 nm	490 nm	Matayoshi, E.D. et al. (1990)
<b>FITC</b> (Fluorescein isothiocyanate)	490 nm	520 nm	Chersi, A. et al. (1990)
<b>Lucifer Yellow</b> (6-Amino-2,3-dihydro-1,3-dioxo-2-hydrazinocarbonylamino-1H-benz[d,e]isoquinoline-5,8-disulfonic acid)	430 nm	520 nm	Grüninger-Leitch, F. et al. (2002)
<b>Mca</b> (7-Methoxycoumarin-4-yl)acetyl)	325 nm	392 nm	Kondo, T. et al. (1997)
<b>Trp</b> (Tryptophan)	280 nm	360 nm	Cezari, M.H. et al. (2002)

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

**Table 2.**  
**Donor/Acceptor Pairs**

Donor (Fluorophore)	Acceptor (Quencher)	References
<b>Abz</b> (2-Aminobenzoyl or Anthraniloyl)	<b>Dnp</b> (2,4-Dinitrophenyl)	Cezari, M.H. et al. (2002)
<b>Abz</b> (2-Aminobenzoyl or Anthraniloyl)	<b>EDDnp</b> (N-(2,4-Dinitrophenyl)ethylenediamine)	Andrau, D. et al. (2003)
<b>Abz</b> (2-Aminobenzoyl or Anthraniloyl)	<b>4-Nitro-Phe</b> (4-Nitro-phenylalanine)	Toth, M.V. and G.R. Marshall (1990)
<b>Abz</b> (2-Aminobenzoyl or Anthraniloyl)	<b>3-Nitro-Tyr</b> (3-Nitro-tyrosine)	Breddam, K. and M. Meldal (1992)
<b>Abz</b> (2-Aminobenzoyl or Anthraniloyl)	<b>pNA</b> (para-Nitroaniline)	Stöckel, A. et al. (1997)
<b>N-Me-Abz</b> (N-Methyl-anthraniloyl)	<b>Dnp</b> (2,4-Dinitrophenyl)	Bickett, D.M. et al. (1993)
<b>Dansyl</b> (5-(Dimethylamino)naphthalene-1-sulfonyl)	<b>4-Nitro-Phe</b> (4-Nitro-phenylalanine)	Florentin, D. et al. (1984)
<b>EDANS</b> (5-[(2-Aminoethyl)amino]-naphthalene-1-sulfonic acid)	<b>DABCYL</b> (4-(4-Dimethylaminophenylazo)benzoyl)	Matayoshi, E.D. et al. (1990)
<b>DMACA</b> (7-Dimethylaminocoumarin-4-acetate)	<b>NBD</b> (7-Nitro-benzo[2,1,3]oxadiazol-4-yl)	Bickett, D.M. et al. (1994)
<b>FITC</b> (Fluorescein isothiocyanate)	<b>Dnp</b> (2,4-Dinitrophenyl)	Korting, H.J. et al. (1977)
<b>Lucifer Yellow</b> (6-Amino-2,3-dihydro-1,3-dioxo-2-hydrazinocarbonylamino-1H-benz[d,e]isoquinoline-5,8-disulfonic acid)	<b>Dabsyl</b> (4-(4-Dimethylaminophenylazo)-benzenesulfonyl)	Grüninger-Leitch, F. et al. (2002)
<b>Mca</b> ((7-Methoxycoumarin-4-yl)acetyl)	<b>Dnp</b> (2,4-Dinitrophenyl)	Kondo, T. et al. (1997)
<b>Trp</b> (Tryptophan)	<b>Dnp</b> (2,4-Dinitrophenyl)	Cezari, M.H. et al. (2002)
<b>Trp</b> (Tryptophan)	<b>4-Nitro-Z</b> (4-Nitro-benzyloxycarbonyl)	Persson, A. and E.B. Wilson (1977)

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**J.R. Lakowicz**

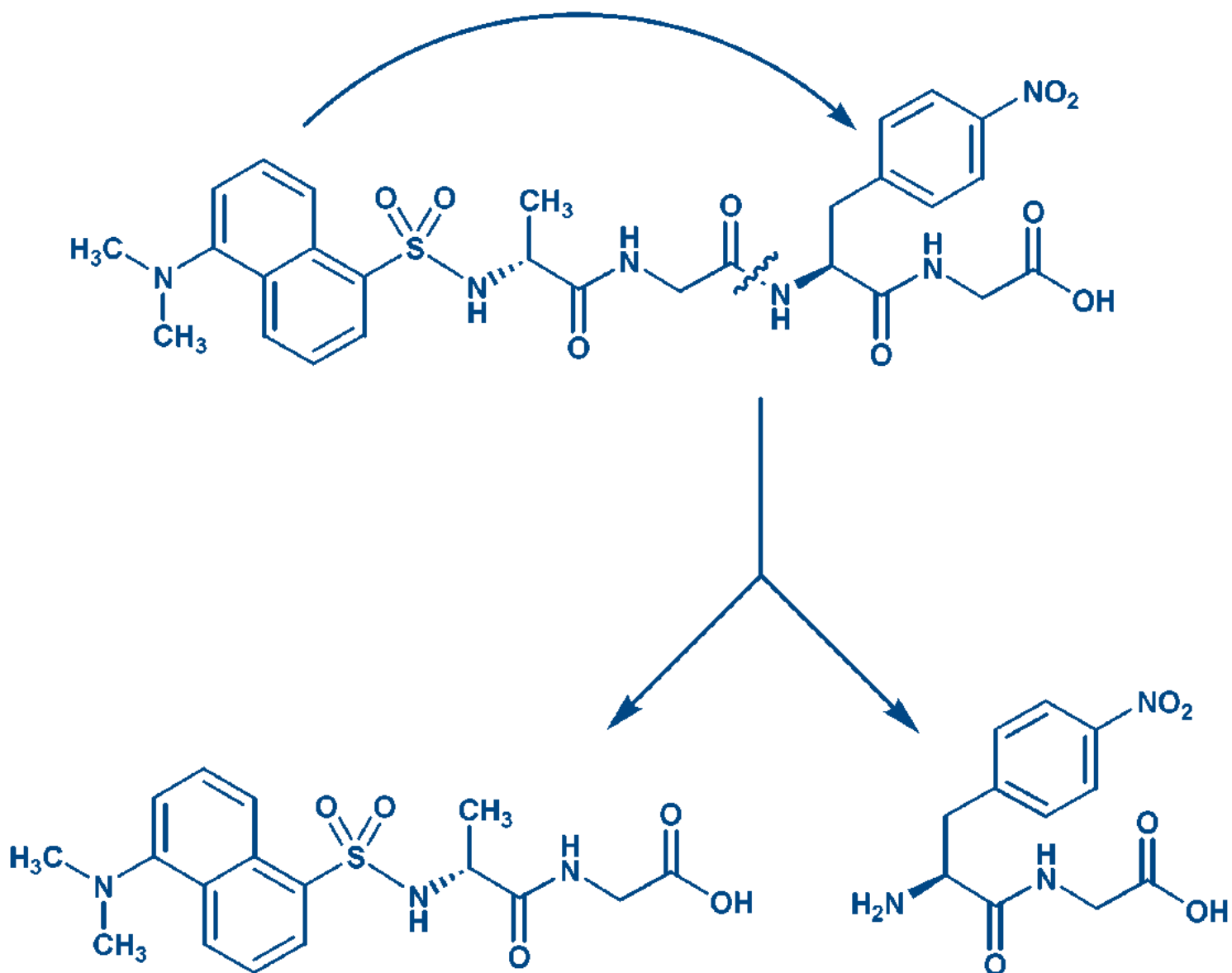
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# FRET SUB- STRATES

FRET Substrates	11-15
Building Blocks for FRET Substrates	16-17

For more information on recommendations on the donor/acceptor pairs in this brochure please see Table 1 and 2 (page 6-7).

## FRET Substrates by Enzyme

Enzyme	Fluorophore / Quencher	Prod. No.
<b>ADAM Protein</b>		
H-Glu(EDANS)-Lys-Pro-Ala-Lys-Phe-Phe-Arg-Leu-Lys(DABCYL)-NH <sub>2</sub>	EDANS/DABCYL	4043075
<b>Aminopeptidase P</b>		
H-Lys(Abz)-Pro-Pro-pNA	Abz/pNA	4027668
<b>Angiotensin I-Converting Enzyme (ACE)</b>		
Abz-Phe-Arg-Lys(Dnp)-Pro-OH	Abz/Dnp	4043877
Abz-Gly-p-nitro-Phe-Pro-OH	Abz/p-nitro-Phe	4003531
<b>Angiotensin-Converting Enzyme 2 (ACE2)</b>		
Abz-Ser-Pro-3-nitro-Tyr-OH	Abz/3-nitro-Tyr	4050533
Mca-Ala-Pro-Lys(Dnp)-OH	Mca/Dnp	4042638
<b>Asp-specific Protease</b>		
Abz-Ala-Phe-Ala-Phe-Asp-Val-Phe-3-nitro-Tyr-Asp-OH	Abz/3-nitro-Tyr	4035170
<b>Calpain-1</b>		
H-Glu(EDANS)-Pro-Leu-Phe-Ala-Glu-Arg-Lys(DABCYL)-OH	EDANS/DABCYL	4050532
<b>Caspase-1</b>		
FITC-Tyr-Val-Ala-Asp-Ala-Pro-Lys(Dnp)-OH (contains FITC isomer I)	FITC/Dnp	4027937
Mca-Tyr-Val-Ala-Asp-Ala-Pro-Lys(Dnp)-OH	Mca/Dnp	4030476

**FRET Substrates by Enzyme (continued)**

Enzyme	Fluorophore / Quencher	Prod. No.
<b>Cathepsin</b>		
Abz-Gly-Ile-Val-Arg-Ala-Lys(Dnp)-OH	Abz/Dnp	4049308
Ac-Glu-Asp(EDANS)-Lys-Pro-Ile-Leu-Phe-Phe-Arg-Leu-Gly-Lys(DABCYL)-Glu-NH <sub>2</sub>	EDANS/DABCYL	4030300
Mca-Gly-Lys-Pro-Ile-Leu-Phe-Phe-Arg-Leu-Lys(Dnp)-D-Arg-NH <sub>2</sub>	Mca/Dnp	4033156
Mca-Gly-Ser-Pro-Ala-Phe-Leu-Ala-Lys(Dnp)-D-Arg-NH <sub>2</sub>	Mca/Dnp	4049855
<b>Cytomegalovirus (CMV) Protease</b>		
DABCYL-Arg-Gly-Val-Val-Asn-Ala-Ser-Ser-Arg-Leu-Ala-EDANS	EDANS/DABCYL	4030469
<b>Endothelin-Converting Enzyme-1</b>		
Mca-Arg-Pro-Pro-Gly-Phe-Ser-Ala-Phe-Lys(Dnp)-OH (Mca-(Ala <sup>7</sup> ,Lys(Dnp) <sup>9</sup> )-Bradykinin)	Mca/Dnp	4029459
<b>Furin</b>		
Abz-Arg-Val-Lys-Arg-Gly-Leu-Ala-m-nitro-Tyr-Asp-OH	Abz/3-nitro-Tyr	4026550
<b>HCV NS3 Protease</b>		
Ac-Asp-Glu-Asp(EDANS)-Glu-Glu-Abu-L-lactoyl-Ser-Lys(DABCYL)-NH <sub>2</sub>	EDANS/DABCYL	4030288
<b>HCV NS3-4A Protease</b>		
Abz-Asp-Asp-Ile-Val-Pro-Cys-Ser-Met-Ser-3-nitro-Tyr-Thr-NH <sub>2</sub>	Abz/3-nitro-Tyr	4050440

Enzyme / Substrate	Fluorophore / Quencher	Prod. No.
<b>HIV Protease</b>		
Abz-Thr-Ile-Nle-p-nitro-Phe-Gln-Arg-NH <sub>2</sub>	Abz/p-nitro-Phe	4030748
DABCYL-γ-Abu-Ser-Gln-Asn-Tyr-Pro-Ile-Val-Gln-EDANS	EDANS/DABCYL	4030716
<b>Kallikrein</b>		
Abz-Ala-Phe-Arg-Phe-Ser-Gln-EDDnp	Abz/EDDnp	4051016

## FRET Substrates by Enzyme (continued)

Enzyme / Substrate	Fluorophore / Quencher	Prod. No.
<b>MMP</b>		
Abz-Lys-Pro-Leu-Gly-Leu-Dap(Dnp)-Ala-Arg-NH <sub>2</sub>		4026336
Dnp-Pro-β-cyclohexyl-Ala-Gly-Cys(Me)-His-Ala-Lys(N-Me-Abz)-NH <sub>2</sub>	N-Me-Abz/Dnp	4025472
6-(7-Nitro-benzo[2,1,3]oxadiazol-4-ylamino)-hexanoyl-Arg-Pro-Lys-Pro-Leu-Ala-Nva-Trp-Lys(7-dimethylaminocoumarin-4-yl)-NH <sub>2</sub>	DMACA/NBD	4028275
DABCYL-γ-Abu-Arg-Pro-Lys-Pro-Val-Glu-Nva-Trp-Arg-Glu(EDANS)-Ala-Lys-NH <sub>2</sub>	EDANS/DABCYL	4037518
DABCYL-γ-Abu-Pro-Gln-Gly-Leu-Glu(EDANS)-Ala-Lys-NH <sub>2</sub>	EDANS/DABCYL	4037519
Mca-Arg-Pro-Lys-Pro-Tyr-Ala-Nva-Trp-Met-Lys(Dnp)-NH <sub>2</sub>	Mca/Dnp	4030841
Mca-Arg-Pro-Lys-Pro-Val-Glu-Nva-Trp-Arg-Lys(Dnp)-NH <sub>2</sub>	Mca/Dnp	4030842
Mca-Arg-Pro-Pro-Gly-Phe-Ser-Ala-Phe-Lys(Dnp)-OH	Mca/Dnp	4029459
Mca-Lys-Pro-Leu-Gly-Leu-Dap(Dnp)-Ala-Arg-NH <sub>2</sub>	Mca/Dnp	4028885
Mca-Pro-β-cyclohexyl-Ala-Gly-Nva-His-Ala-Dap(Dnp)-NH <sub>2</sub>	Mca/Dnp	4038444
Mca-Pro-Leu-Ala-Cys(Mob)-Trp-Ala-Arg-Dap(Dnp)-NH <sub>2</sub>	Mca/Dnp	4038699
Mca-Pro-Leu-Ala-Nva-Dap(Dnp)-Ala-Arg-NH <sub>2</sub>	Mca/Dnp	4040959
Mca-Pro-Leu-Gly-Leu-Dap(Dnp)-Ala-Arg-NH <sub>2</sub>	Mca/Dnp	4026036
Mca-Pro-Leu-Gly-Leu-Glu-Glu-Ala-Dap(Dnp)-NH <sub>2</sub>	Mca/Dnp	4053570
Mca-Pro-Lys-Pro-Leu-Ala-Leu-Dap(Dnp)-Ala-Arg-NH <sub>2</sub>	Mca/Dnp	4030807
Dnp-Arg-Pro-Leu-Ala-Leu-Trp-Arg-Ser-OH	Trp/Dnp	4030541
Dnp-Pro-Leu-Gly-Leu-Trp-Ala-D-Arg-NH <sub>2</sub>	Trp/Dnp	4018011
<b>Neprilysin</b>		
Dansyl-D-Ala-Gly-4-nitro-Phe-Gly-OH	Dansyl/4-nitro-Phe	4050412
<b>Neutral Metalloendopeptidase</b>		
Abz-Ala-Gly-Leu-Ala-p-nitrobenzylamide	Abz/p-nitrobenzylamide	4014232
<b>Papain</b>		
Abz-Gln-Val-Val-Ala-Gly-Ala-ethylenediamine-Dnp	Abz/EDDnp	4026185
<b>Renin</b>		
DABCYL-γ-Abu-Ile-His-Pro-Phe-His-Leu-Val-Ile-His-Thr-EDANS	EDANS/DABCYL	4031169

Enzyme / Substrate	Fluorophore / Quencher	Prod. No.
<b>SARS Main Protease</b>		
DABCYL-Lys-Thr-Ser-Ala-Val-Leu-Gln-Ser-Gly-Phe-Arg-Lys-Met-Glu-EDANS	EDANS/DABCYL	4045664
<b>β-Secretase</b>		
Abz-Val-Asn-Leu-Asp-Ala-Glu-EDDnp	Abz/EDDnp	4045326
Abz-Val-Lys-Met-Asp-Ala-Glu-EDDnp	Abz/EDDnp	4045325
H-Arg-Glu(EDANS)-Glu-Val-Asn-Leu-Asp-Ala-Glu-Phe-Lys(DABCYL)-Arg-OH	EDANS/DABCYL	4033536
Mca-Ser-Glu-Val-Asn-Leu-Asp-Ala-Glu-Phe-Arg-Lys(Dnp)-Arg-Arg-NH <sub>2</sub>	Mca/Dnp	4033760
Mca-Ser-Glu-Val-Asn-Leu-Asp-Ala-Glu-Phe-Lys(Dnp)-OH	Mca/Dnp	4029476
Mca-Ser-Glu-Val-Asn-Leu-Asp-Ala-Glu-Phe-Lys(Dnp)-NH <sub>2</sub>	Mca/Dnp	4034744
Mca-Ser-Glu-Val-Lys-Met-Asp-Ala-Glu-Phe-Arg-Lys(Dnp)-Arg-Arg-NH <sub>2</sub>	Mca/Dnp	4033759
<b>γ-Secretase</b>		
Abz-Gly-Gly-Val-Val-Ile-Ala-Thr-Val-Lys(Dnp)-D-Arg-D-Arg-D-Arg-NH <sub>2</sub>	Abz/Dnp	4043077
N-Me-Abz-Gly-Gly-Val-Val-Ile-Ala-Thr-Val-Lys(Dnp)-D-Arg-D-Arg-D-Arg-NH <sub>2</sub>	N-Me-Abz/Dnp	4043236
<b>Thimet Oligopeptidase</b>		
Mca-Pro-Leu-Gly-Pro-D-Lys(Dnp)-OH	Mca/Dnp	4027687
<b>TNF-α Converting Enzyme (TACE, ADAM17 endopeptidase)</b>		
Mca-(endo-1a-Dap(Dnp))-TNF-α (-5 to +6) amide (human)		4031302
DABCYL-Leu-Ala-Gln-Ala-Val-Arg-Ser-Ser-Ser-Arg-EDANS	EDANS/DABCYL	4031301
H-Arg-Glu(EDANS)-Glu-Val-Asn-Leu-Asp-Ala-Glu-Phe-Lys(DABCYL)-Arg-OH	Mca/Dnp	4033536

## Building Blocks for FRET Substrates

Building Block	Prod. No.
<b>Dabsyl Derivatives</b>	
Fmoc-Lys(Dabsyl)-OH	4050342
<b>Dnp Derivatives</b>	
Fmoc-Dap(Dnp)-OH	4026365
Fmoc-Dap(Dnp)-Sasrin™ resin	4029525
Fmoc-Lys(Dnp)-OH	4026832
Fmoc-D-Lys(Dnp)-OH	4027754
Fmoc-Orn(Dnp)-OH	4030622
Dnp-Pro-OH	4008147
<b>4-Nitrophenylalanine (a choice of derivatives)</b>	
Boc-p-nitro-Phe-OH	4001953
Boc-p-nitro-D-Phe-OH	4001979
Fmoc-p-nitro-Phe-OH	4010331
Fmoc-p-nitro-D-Phe-OH	4019048
<b>3-Nitro- and 3,5-Dinitrotyrosine</b>	
Fmoc-3-nitro-Tyr-OH	4026244



Fluorophores	Prod. No.
<b>Abz</b>	
Boc-Abz-OH	4002711
Boc-N-Me-Abz-OH	4025576
Fmoc-Abz-OH	4028486
Fmoc-Lys(retro-Abz-Boc)-OH	4027855
<b>Dansyl and EDANS</b>	
Fmoc-Lys(dansyl)-OH	4026510
Fmoc-Asp(EDANS)-OH	4049431
Fmoc-Glu(EDANS)-OH	4033590
<b>Fluorescein and Rhodamine</b>	
5-Carboxy-fluorescein	4045141
6-Carboxy-fluorescein	4045142
5(6)-Carboxy-tetramethylrhodamine (TAMRA) (used in combination with FAM)	4045146
<b>Mca</b>	
(7-Methoxycoumarin-4-yl)acetic acid (Mca-OH)	4026019
Fmoc- $\beta$ -(7-methoxy-coumarin-4-yl)-Ala-OH	4046868
<b>Tryptophan (a choice of derivatives)</b>	
Boc-Trp-OH	4000240
Boc-Trp(For)-OH	4001264
Fmoc-Trp-OH	4003185
Fmoc-Trp(Boc)-OH	4017674

## FRET Substrate Cleavage Products

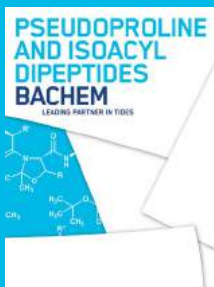
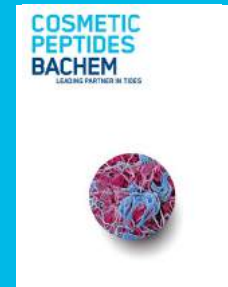
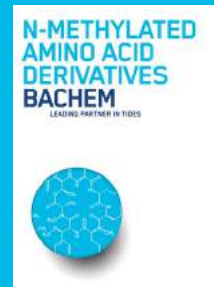
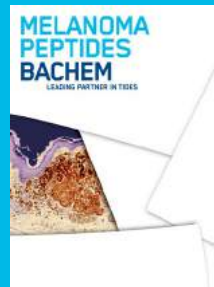
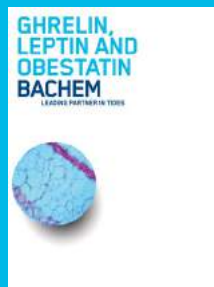
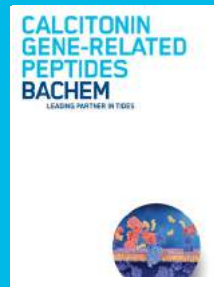
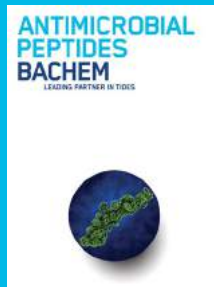
Cleavage Product	Substrate	Prod. No.
Abz-Gly-OH · HCl	Abz-Gly-p-nitro-Phe-Pro-OH (4003531)	4015644

### Custom Peptide Synthesis

- A strong commitment to quality is the basis of our long-standing market leadership
- Almost 50 years of peptide experience with facilities in the USA and Europe
- Highly motivated and experienced team to help with your sequence design and modifications
- Capacity to produce short to complex peptides from mg to multi-kg and beyond
- Cited as source in over 13,000 world wide science publications  
(Highwire Press, [www.highwire.org](http://www.highwire.org))

Although Bachem offers a variety of FRET substrates and related compounds from stock as catalog products, your project may require a substrate not listed in our catalog. Take advantage of our expertise and contact our custom peptide service at [www.bachem.com](http://www.bachem.com). Our experts will support you with the design of your substrate.

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