FRET

TECHNOLOGY

The dawn of long wavelength protease FRET assays

ANASPEC

ISO 9001:2000 Company

2nd Edition

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AnaSpec, the leader in peptide FRET technology

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- **Industry's first long-wavelength assays:**
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- **Homogeneous assays**
- **High sensitivity**
- **Minimal autofluorescence**

Fluorescence Resonance Energy Transfer (FRET) - Introduction

Fluorescence or Förster resonance energy transfer (FRET) is a distance-dependent transfer of excited state energy from an initially excited donor to an acceptor, with the donor molecule typically emitting at shorter wavelengths that overlap with the absorption of an acceptor (1-3). FRET occurs when a donor (fluorophore) and an acceptor (another fluorophore or quencher) are within a specified distance, usually within 10-100 Å. The donor-acceptor distance at which the energy transfer is 50% is called the Förster radius (R_o). Within this distance, when a donor transfers its resonance energy to a quencher, a decrease in the donor fluorescence is seen. FRET efficiency falls dramatically as the donor-acceptor distance exceeds the Förster radius. Figure 1 shows a schematic representation of a FRET peptide where the fluorescence of the donor is quenched through resonance energy transfer. Enzyme hydrolysis of the peptide results in spatial separation of the donor and acceptor, which leads to the recovery of the fluorescence of the donor.

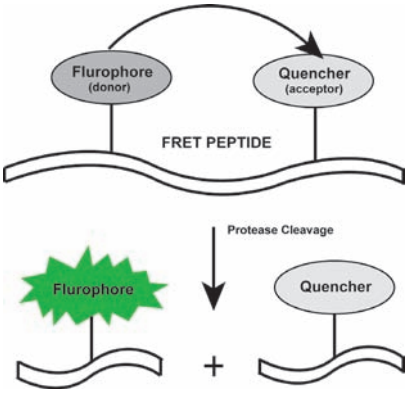

Figure 1. Schematic representation of a FRET peptide proteolytic cleavage.

Biological Application of FRET – Protease Detection

In recent years, FRET based assays have found broad applications, one of which is the detection of proteases. Proteases, also known as peptidases, are enzymes which catalyze the hydrolysis of peptide bonds (4). Ubiquitously distributed in all tissues and biological fluids, they play key roles in protein activation, cell regulation and signaling, as well as in the generation of amino acids for protein synthesis or utilization in metabolic pathways (5-7). Proteases also play important roles in the pathogenesis of several major diseases. The discovery that an HIV protease inhibitor was effective in the treatment of AIDS (8) has spurred similar research for the identification of protease involvement in other diseases; and more importantly, research into potential protease inhibitors.

Numerous methods are used in the analysis of proteases present in solutions, cells or tissues; however, spectrophotometric method has been favored due to its high speed, better accuracy and ease of use. This method has been predominantly used in high throughput screening (HTS) of protease activities and inhibitors. The spectral and enzymatic properties of chromogenic and fluorogenic substrates play a critical role in the successful use of spectrophotometric methods for analyzing proteases. In general, fluorogenic substrates are several orders of magnitude more sensitive than chromogenic substrates, they have a wide linear dynamic range and offer good reproducibility (9). In recent years, FRET-based assays have been used extensively in the detection of different proteases, which made the continuous assay of protease activity and HTS of protease inhibitors faster and easier.

AnaSpec, a world leader in FRET peptide technology, is proud to be the first in the industry to offer a variety of long wavelength FRET based assay kits and/or substrates for use in drug discovery research. Compared to the traditional shorter wavelength FRET pair, DABCYL/EDANS, these long wavelength substrates exhibit better sensitivity. At higher excitation and emission (Ex/Em) wavelengths, interference from autofluorescence of cellular components and test compounds is minimal. For example, the 5-FAM/QXL™ 520 renin substrate is 40-fold more sensitive than a DABCYL/EDANS FRET substrate (see p. 10). In this brochure, we feature long wavelength protease assay kits for Cathepsins D, S; HCV; HIV; MMPs; α, β-Secretase; Renin and WNV; as well as a shorter wavelength assay for ACE2. Information of two generic protease assays, which employ the same fluorophore as both the donor and quencher, is also included.

References:

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3. Dietrich, A. et al. *J. Biotechnol.* **82**, 211 (2002).
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7. Clawson, GA. *Cancer Invest.* **14**, 597 (1996).
8. Ho, DD. et al. *Nature* **373**, 123 (1995).
9. Patton, WF. *J. Chromotography B.* **771**, 2 (2002).

Cathepsin D Activity Assay Kits

Cathepsins are a class of globular lysosomal proteases, playing a vital role in mammalian cellular turnover. They degrade polypeptides and are distinguished by their substrate specificities. Cathepsin D is the lysosomal aspartic proteinase implicated in intracellular protein degradation. It is involved in several pathological processes, such as inflammatory states, atherosclerosis, thrombosis, apoptosis, neoplastic proliferation and Alzheimer disease (1-5).

The SensoLyte™ 520 Cathepsin D Activity Assay Kit is a homogeneous assay that can be used to detect Cathepsin D activity in biological samples or in purified enzyme preparations. A unique long wavelength FRET substrate was designed based on a sequence surrounding the cleavage site of Cathepsin D (6, 7). This 5-FAM/QXL™ 520 FRET pair shows optimal quenching of the intact substrate. When active Cathepsin D cleaves this FRET substrate, it results in an increase of 5-FAM fluorescence, which can be monitored at Ex/Em = 490 nm/520 nm. The long wavelength fluorescence of 5-FAM shows less interference from autofluorescence of cellular components and test compounds.

	SensoLyte™ 520 Cathepsin D Assay Kit Cat# 72097
FRET pair	5-FAM/QXL™ 520
Ex/Em (nm/nm)	490/520

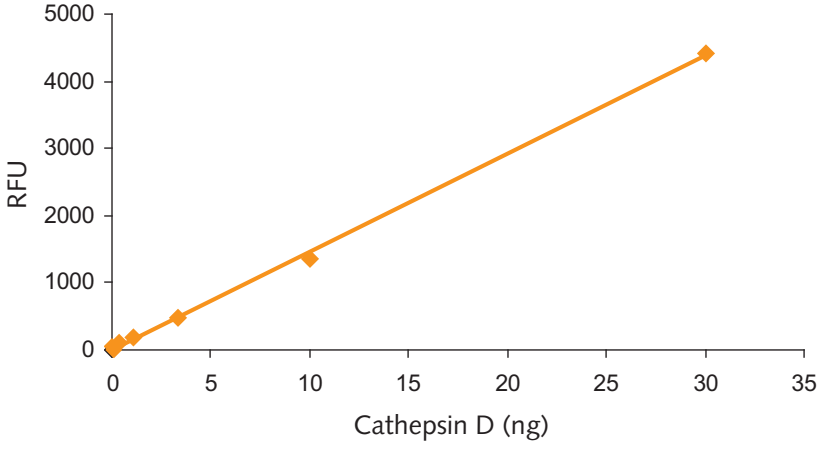

Figure 2. Detection of Cathepsin D using the SensoLyte™ 520 Cathepsin D Assay Kit. The kit can detect as low as 0.5 ng (0.2 mUnits) Cathepsin D.

Shorter Wavelength Assay Kit

SensoLyte™ 390 Cathepsin D Assay Kit, cat# 72098. This kit provides a convenient assay for screening of Cathepsin D enzyme inhibitors and for continuous assay of Cathepsin D activity using a Mca/Dnp FRET peptide. In the intact FRET peptide, the fluorescence of Mca is quenched by Dnp. Upon cleavage into two separate fragments by Cathepsin D, the fluorescence of Mca is recovered, and can be monitored at Ex/Em = 330 nm/390 nm.

Related Products

Anti-Cathepsin D, cat# 53304

SensoLyte™ 520 α-Secretase or TACE Assay Kit (Ex/Em=490 nm/520 nm) - cat# 72085.

SensoLyte™ 520 β-Secretase Assay Kit (Ex/Em=490 nm/520 nm) - cat# 71144.

β-Amyloids and related peptides - AnaSpec is the world's largest provider of β-Amyloids and related peptides. Search online under Peptides>Catalog Peptides>Amyloids.

References:

1. Berchem, G. et al. *Oncogene* **21**, 5951(2002).
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3. Davidson, Y. et al. *J. Neurol. Neurosurg. Psychiat.* **77**, 515 (2006).
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Cathepsin S Activity Assay Kits

Cathepsin S is a lysosomal cysteine proteinase of the papain family. It plays a major role in the degradation of the invariant peptide chain associated with the major histocompatibility complex and thus affects antigen presentation (1, 2). Since antigen presentation is the key to immune response, Cathepsin S has been validated as an immunomodulatory target (3, 4). It is also involved in several pathologies including atherosclerosis, cancer, obesity and the associated diseases (5-9).

The SensoLyte™ 520 Cathepsin S Activity Assay Kit is a homogeneous assay that can be used to detect Cathepsin S activity in biological samples or in purified enzyme preparations. A unique 5-FAM/QXL™ 520 FRET peptide substrate is used in this kit. When active Cathepsin S cleaves this FRET substrate, it results in an increase of 5-FAM fluorescence, which can be monitored at Ex/Em = 490 nm/520 nm. The long wavelength fluorescence of 5-FAM shows less interference from autofluorescence of cellular components and test compounds.

	SensoLyte™ 520 Cathepsin S Assay Kit Cat# 72099
FRET pair	5-FAM/QXL™ 520
Ex/Em (nm/nm)	490/520

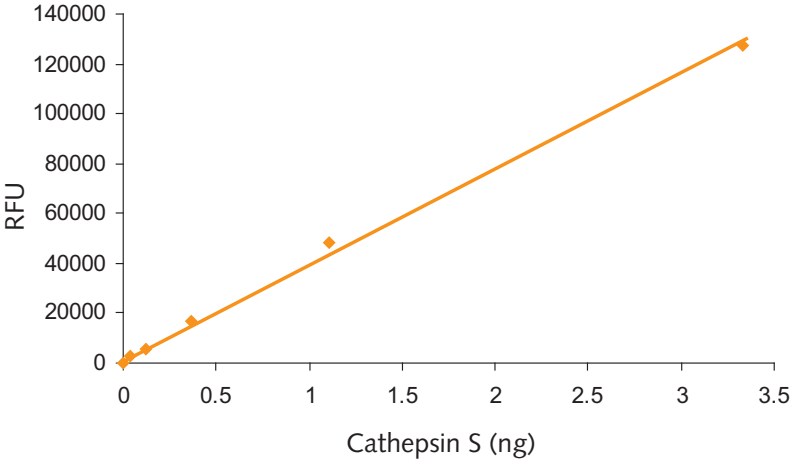

Figure 3. Detection of Cathepsin S using the SensoLyte™ 520 Cathepsin S Assay Kit. The kit can detect 0.1 ng (0.03 mUnits) Cathepsin S.

Shorter Wavelength Assay Kit

SensoLyte™ 440 Cathepsin S Assay Kit, cat# 72100. This kit provides a convenient assay for screening Cathepsin S enzyme inhibitors and for continuous assay of enzyme activity using a fluorogenic peptide specific for Cathepsin S. Upon Cathepsin S cleavage, the AMC (7-amino-4-methylcoumarin) fluorophore is released. AMC has bright blue fluorescence which can be monitored at Ex/Em=354 nm/442 nm.

Related Products

SensoLyte™ 520 α-Secretase or TACE Assay Kit (Ex/Em=490 nm/520 nm) - cat# 72085.

SensoLyte™ 520 β-Secretase Assay Kit (Ex/Em=490 nm/520 nm) - cat# 71144.

β-Amyloids and related peptides - AnaSpec is the world's largest provider of β-Amyloids and related peptides. Search online under Peptides>Catalog Peptides>Amyloids.

References:

1. Honey, K and AY. Rudensky, *Nat. Rev. Immunol.* **3**, 472 (2003).
2. Hsieh, CS. et al. *J. Immunol.* **168**, 2618 (2002).
3. Gupta, S. et al. *Expert Opin. Ther. Targets* **12**, 291 (2008).
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HCV (Hepatitis C Virus) Assay Kits

Hepatitis C virus (HCV), discovered in 1989, infects approximately 170 million people worldwide (1, 2). It belongs to the *Flaviviridae* family of positive, single stranded RNA, and its polyprotein consists of structural proteins (C, E1, E2 and p7), and the non-structural (NS) proteins (NS2, NS3, NS4A, NS4B, NS5A, and NS5B), resulting from proteolytical cleavages of host signal peptidases; metalloprotease and serine proteases, respectively (3-6). The protease responsible for the cleavage of HCV non-structural polyprotein at the NS3-NS4A, NS4A-NS4B, NS4B-NS5A, and NS5A-NS5B sites is the NS3/4A protease. Since these cleavages are essential for the maturation of the viral proteins, this protease has become one of the key targets for developing anti-HCV drugs (7-11).

Applying both peptide and fluorescent dye technologies, AnaSpec has developed an ultra-sensitive HCV NS3/4A protease FRET substrate that can detect the presence of HCV NS3/4A protease at levels of at least 0.01 pmol. Based on an EDANS/DABCYL substrate described by Taliani, M. et al. (12), the donor and the quencher were substituted with 5-FAM and our trademark quencher, QXL™ 520. This substrate, found in SensoLyte™ 520 HCV Protease Assay Kit (Cat# 71145), shows a 22-fold lower K*m* compared to the EDANS/DABCYL peptide, the substrate found in SensoLyte™ 490 HCV Protease Assay Kit (Cat# 71126 and 72087). These kits and the SensoLyte™ 620 kit have been developed by AnaSpec for HTS screening of inhibitors and for measuring HCV NS3/4A activity (Table 1).

Table 1. Comparison of the three fluorimetric SensoLyte™ HCV protease assay kits.

	SensoLyte™ 490 HCV Protease Assay Kit Cat# 71126, 72087	SensoLyte™ 520 HCV Protease Assay Kit Cat# 71145	SensoLyte™ 620 HCV Protease Assay Kit Cat# 71146
FRET pair	EDANS/DABCYL	5-FAM/QXL™ 520	HiLyte Fluor™ TR/QXL™ 610
Ex/Em (nm/nm)	340/490	490/520	590/620

Table 2. Kinetic comparison of two HCV substrates.

	EDANS/DABCYL FRET Substrate	5-FAM/QXL™ 520 FRET Substrate
K*m* (uM)	69.4	3.2
K*cat* (min^{-1})	16.5	2.7
K*cat*/K*m* ($M^{-1}s^{-1}$)	3961.0	14127.3

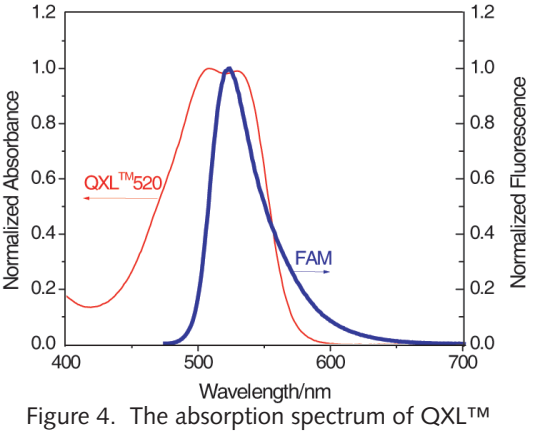

Figure 4. The absorption spectrum of QXL™ 520 perfectly overlaps with the emission spectrum of 5-FAM.

Related Products

HCV NS3/4A Protease Substrate, Ac-DE-Dap(QXL™ 520)-EE-Abu-ψ[COO]ASC(5-FAMsp)-NH_2 (Ex/Em=490 nm/520 nm) - cat# 60798 (1 mg).

HCV Protease Substrate (RET S1), Ac-DED(EDANS)EE-Abu-ψ[COO]ASK(DABCYL)-NH_2 (Ex/Em=340 nm/490 nm) - cat# 22991-025 (0.25 mg), 22991 (1 mg).

Hydrolysis products of the above substrates - for use as controls
HCV NS3/4A Protease Substrate Hydrolysis Products 1 & 2 - cat# 60978 and 60979.
RET S1 - cat# 25334 and 25132.

Recombinant HCV NS3/4A proteases (5 and 10 ug sizes):
Wild-type - cat# 61017-5 (5 ug) and cat# 61017-10 (10 ug).
Mutants (single amino acid substitutions) - cat# 72022, 72023, 72031 to 72037.

Peptide fragments of HCV polyprotein: Search online under Peptides>Catalog Peptides>HCV-Related Peptides.

NS3 Protease Inhibitors - cat# 25345-25348.

References:

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3. Choo, QL. et al. *Proc. Natl. Acad. Sci. USA* **88**, 2451 (1991).
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HIV (Human Immunodeficiency Virus) Assay Kits

The 10 -12 kD aspartic protease of human immunodeficiency virus-1 (HIV-1) is essential for post-translational cleavage of HIV precursor polyproteins, Pr^{gag} and $Pr^{gag\text{-}pol}$ (1, 2). Since these cleavages are essential for the maturation of the infectious virus, this protease has become one of the key targets for developing anti-AIDS drugs (3). AnaSpec has developed three assay kits for measuring the activity of this protease and for HTS of its inhibitors. In each of the three SensoLyte™ HIV assay kits, a substrate containing a different FRET pair is used (Table 3). The general sequence of the peptide is derived from the native p17/p24 cleavage site on Pr^{gag} for HIV-1 protease. Incubation of recombinant HIV-1 protease with the substrate results in specific cleavage and a time-dependent increase in fluorescence intensity that is linearly-related to the extent of substrate hydrolysis. Due to its simplicity and precision in the determination of reaction rates required for kinetic analysis, these assays, offer many advantages over the commonly used HPLC or electrophoresis-based assays.

Table 3. Comparison of the three fluorimetric SensoLyte™ HIV protease assay kits.

	SensoLyte™ 490 HIV Protease Assay Kit Cat# 71127	SensoLyte™ 520 HIV Protease Assay Kit Cat# 71147	SensoLyte™ 620 HIV Protease Assay Kit Cat# 71148
FRET pair	EDANS/DABCYL	HiLyte Fluor™ 488/QXL™ 520	HiLyte Fluor™ TR/QXL™ 610
Ex/Em (nm/nm)	340/490	490/520	590/620

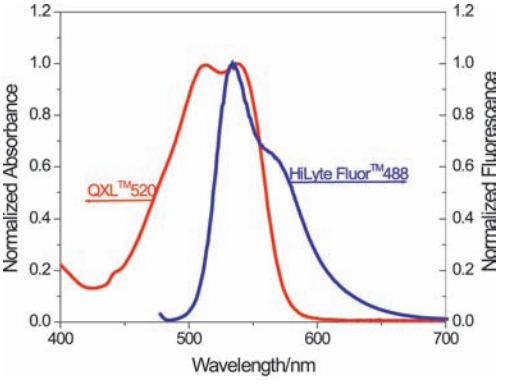

Figure 5. The absorption spectrum of QXL™ 520 perfectly overlaps with the emission spectrum of HiLyte Fluor™ 488.

Table 4. Kinetic comparison of two HIV substrates.

	EDANS/DABCYL FRET Substrate	HiLyte Fluor™/QXL™ 520 FRET Substrate
Km (uM)	11.6	6.912
Kcat/*Km* ($M^{-1}s^{-1}$)	$5.3x10^5$	$1.7x10^7$
Vmax (RFU/sec)	0.38	7.12

Related Products

HIV Protease FRET Substrate, DABCYL-GABA-SQNYPIVQ-EDANS (Ex/Em=340 nm/490 nm) - cat# 22992 (1 mg).

Recombinant HIV NS3/4A Protease: Wild-Type - cat# 72028-5.

Peptides: AnaSpec provides over 5000 HIV-related peptides, including gag, env, TAT, pol and more.
Search online under Peptides>Catalog Peptides>HIV-Related Peptides.

References:

1. Seelmeier, S. et al. *Proc. Natl. Acad. Sci. USA* **85**, 6612 (1988).
2. Schneider, J. and SBH. Kent, *Cell* **54**, 363 (1988).
3. Gehringer, H. et al. *J. Virol. Methods* **109**, 143 (2003).

MMP (Matrix Metalloproteinases) Assay Kits

Matrix metalloproteinases (MMPs) are a family of highly homologous protein-degrading zinc dependent enzymes endopeptidases.This family currently includes more than 25 members that can be divided into collagenases (MMP-1, -8, and -13), gelatinases (MMP-2 and 9), stromelysins (MMP-3 and 10), matrilysins (MMP-7 and 26), and the membrane-type MMPs (MMP-14 to 17 and 24). MMPs are important in many normal biological processes including embryonic development, angiogenesis, and wound healing, as well as in pathological processes such as inflammation, cancer, and tissue destruction. MMPs collectively cleave most, if not all, of the constituents of the extracellular matrix (ECM) and are involved in the breakdown and remodeling of many tissues and organs (1-3).

AnaSpec is proud to introduce the next level in MMP detection. SensoLyte™ fluorimetric MMP assay kits use AnaSpec's proprietary FRET substrates to accurately detect activity in a wide range of MMPs. These kits are robust (mix-and-read format) and highly sensitive. They are ideal for the detection of MMP activity in a variety of biological samples, including synovial fluids, plasma, conditioned culture mediums, cells or tissue extracts. The whole assay takes less than 10 minutes hands-on time and provides results in less than 30 minutes. Our 5-FAM/QXL™ 520-based FRET assay kits have demonstrated greatly improved performance over existing MMP assay kits which use either the less sensitive EDANS/DABCYL FRET substrates or colorimetric detection. These SensoLyte™ 520 assay kits use the FRET substrates that incorporate our QXL™ 520 non-fluorescent dye, the best quencher available for 5-FAM. These novel MMP assay kits are widely used for screening MMP activities or MMP inhibitors.

The SensoLyte Plus™ 520 MMP assay kits are designed for the detection of a specific MMP in a biological mixed sample which may contain different MMPs. A monoclonal human-MMP antibody is used to pull down both the pro and active forms of an MMP from the mixture, and its proteolytic activity is quantitated using a 5-FAM/QXL™ 520 FRET peptide (Figure 6).

	SensoLyte™ 490 Series Ex/Em=340 nm/490 nm Cat#	SensoLyte™ 520 Series Ex/Em=490 nm/520 nm Cat#	SensoLyte Plus™ 520 Series Ex/Em=490 nm/520 nm Cat#	Recombinant Human MMPs Cat#
MMP-1	71128	71150	72012	72004
MMP-2	71129	71151		72005
MMP-3	71130	71152		72006
MMP-7	71132 (Ex/Em=370 nm/460 nm)	71153		72007
MMP-8	71133	71154		72008
MMP-9	71134	71155	72017	72009 72069 (mouse)
MMP-10		72024		72067
MMP-12	71137	71157		72010
MMP-13	71135	71156	72019	72011
MMP-14		72025		72068

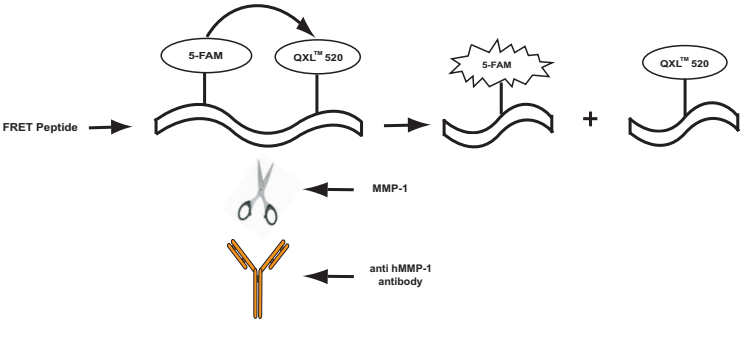

Figure 6. Schematic representation of the principle behind the SensoLyte Plus™ 520 MMP Assay Kits. The example shown here is from the SensoLyte Plus™ 520 MMP-1 Assay Kit. MMP-1 in biological samples is first captured by an immobilized human MMP-1 monoclonal antibody. Proteolytic activity of the captured MMP-1 cleaves the FRET substrate and is monitored at Ex/Em=490±20 nm/520±20 nm.

MMP (Matrix Metalloproteinases) Assay Kits (con't)

SensoLyte™ Generic MMP Assay Kits

The SensoLyte™ Generic MMP Assay Kits are optimized to detect the activity of a variety of MMPs (see table below). The SensoLyte™ 520 Generic MMP Assay Kit employs a green fluorophore (5-FAM) and the SensoLyte™ 570 Generic MMP, a red fluorophore (5-TAMRA). These assay kits are ideal for detecting generic MMP activity in biological samples or for high throughput screening of MMP inducers and inhibitors using purified MMPs.

	SensoLyte™ 520 Generic MMP Assay Kit Cat# 71158	SensoLyte™ 570 Generic MMP Assay Kit Cat# 72101
FRET pair	5-FAM/QXL™ 520	5-TAMRA/QXL™ 570
Ex/Em (nm/nm)	490/520	540/575
Cleaved by	MMP-1, 2, 3, 7, 8, 9, 12, 13, and 14	MMP-1, 2, 7, 8, 9, 10, 13, and 14

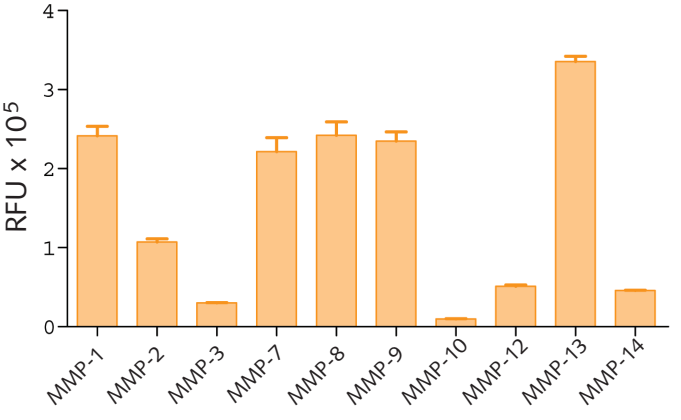

Figure 7. The SensoLyte™ 520 Generic Assay Kit detects the activity of several MMPs. APMA-activated MMPs, at 30 ng each, were incubated with 5-FAM/QXL™ 520 FRET substrate. Fluorescence was monitored 1h after the start of the reaction (Ex/Em=490 nm/520 nm).

Other MMP Assay Kits

SensoLyte™ 520 MMP Profiling Kit *Fluorimetric*- two 96-well plates coated with 16 MMP substrates - cat# 71136.
SensoLyte™ 520 MMP Substrate Sampler Kit *Fluorimetric*- contains 16 MMP individually packed substrates - cat# 71170.
SensoLyte™ Generic MMP Assay Kit *Colorimetric*(Abs = 412 nm) - cat# 72095.

Table 5. AnaSpec's SensoLyte™ MMP assay kits have been validated using purified human MMPs; however, as the list below shows, MMPs from various sources have been used and reported by researchers.

Cat#	Assay Kit	Application
71150	SensoLyte™ 520 MMP-1 Assay Kit	MMP-1 activity measured in hyperhomocysteinemic rats. *Yi, F. et al. Kidney Int. 70, 88 (2006).*
71151	SensoLyte™ 520 MMP-2 Assay Kit	(human) pro-MMP-2 … first activated with 1 mm APMA for 20 min or 2 h… *Johnson, SL. et al. Chem. Bio. Drug Design 71, 131 (2008); Johnson, SL. et al. Bioorganic Chem. 35, 306 (2007).* …tissue extracts were obtained from nonpregnant, pregnant, and postpartum mice…and a fluorescently labeled substrate specific for MMP2 was used to determine enzyme activity. *Wieslander, C. et al. Biol. Reprod.. 10.1095/biolreprod.107.063024 (2007).*
71152	SensoLyte™ 520 MMP-3 Assay Kit	...activity in the astrocytic culture medium was determined using a stromelysin-1 (MMP-3) activity assay kit (SensoLyte 520 MMP-3 assay kit)… *Koyama, Y. and T. Tanaka, BBRC, doi:10.1016/j.bbrc.2008.04.064 (2008).*
71155	SensoLyte™ 520 MMP-9 Assay Kit	(human) … pro-MMP-9 … first activated with 1 mm APMA for 20 min or 2 h… *Johnson, SL. et al. Chem. Bio. Drug Design 71, 131 (2008); Johnson, SL. et al. Bioorganic Chem. 35, 306 (2007).*
71156	SensoLyte™ 520 MMP-13 Assay Kit	MMP-13 (rat interstitial collagenase) activity was detected at 0 and 7 days using the SensoLyte™ (formerly Enzolyte) 520 MMP-13 assay kit. *Arnoczky, SP. et al. Am. J. Sports Med. 35, 763 (2007).*
72017	SensoLyte Plus™ 520 MMP-9 Assay Kit	MMP-9 activities of cell (MiaPaCa2 and PANC1 human pancreatic ductal adenocarcinoma cells) or tumor lysates were assessed using the SensoLyte (formerly Enzolyte) Plus™ 520 Enhanced Selectivity MMP-9 activity. *Liau, S-S. et al. Cancer Res. 66, 11613 (2006).* … genetically altered cells (esophageal squamous) were used to detect MMP-9 enzymatic activity in duplicate using the SenzoLyte Plus MMP-9 assay kit from AnaSpec. Okawa, *T. et al. Genes & Dev. 21, 2788 (2007).*
72019	SensoLyte Plus™ 520 MMP-13 Assay Kit	MMP-13 activity (in RKO- human colorectal carcinoma cell line) using the SensoLyte Plus™ 520 MMP-13 Assay. *Bai, L. et al. FEBS Lett. 581, 5904 (2007).*
71158	SensoLyte™ 520 Generic MMP Assay Kit	General MMP activity (from rat heart) was measured using the SensoLyte™ (formerly Enzolyte) 520 Generic MMP assay kit. *Errami, M. et al. J. Pharma. Exp. Ther. DOI: 10.1124/jpet.107.133975 (2007).* SensoLyte™ 520 Generic MMP assays were used to determine overall activity of MMPs in coronin-3 knockdown in U373 and A172 human glioblastoma cells. *Thal, DR. et al. J. Pathol. 214, 415 (2008).*
71170	SensoLyte™ 520 MMP Substrate Sampler Kit	*Aspergillus* culture supernatants, human serum and the mixture of both..(using)..fluorescence resonance energy transfer (FRET) were chosen out of a commercially available protease assay kit comprising 16 different substrates SensoLyte™ (formerly Enzolyte) 520 matrixmetalloprotease (MMP) substrate sample kit, AnaSpec... *Schaal, R. et al. J. Microbio. Meth. 71, 93 (2007).* The activity of MMPs in the conditioned medium and cell lysates (CHO or APP-overexpressed neuroblastoma cells) was determined using SensoLyte™ (formerly EnzoLyte) MMP fluorometric assay kit. *White, AR. et al. J. Biol. Chem. 281, 17670 (2006).*

360 MMP FRET Substrates

These MMP peptide substrates use Trp/Dnp as the FRET pair. In the intact peptide substrate, Dnp quenches the fluorescence of Trp (W). Proteolytic cleavage of the FRET peptide separates the fluorophore and quencher, thus recovering the quenched fluorescence of Trp. The released fluorescence is directly related to MMP activities and is monitored at Ex/Em=280 nm/360 nm (9-15).

Cat#	Product Name	Sequence	Cleaved by
27074, 27075	360 MMP FRET Substrate I	Dnp-PLGLWAr-NH_2	N.D.*
27082, 27083	360 MMP FRET Substrate II	Dnp-PLALWAR	MMP-1
27094, 27095	360 MMP FRET Substrate III	Dnp-PLGMWSR	MMP-2/9
27098, 27099	360 MMP FRET Substrate IV	Dnp-PYAYWMR	MMP-3
27102, 27103	360 MMP FRET Substrate V	Dnp-RPLALWRS	MMP-7
27106, 27107	360 MMP FRET Substrate VI	Dnp-PLAYWAR	MMP-8

390 MMP FRET Substrates

These MMP peptide substrates use Mca/Dnp as FRET pair. In the intact peptide substrate, Dnp quenches the fluorescence of Mca. Proteolytic cleavage of FRET peptide separates the fluorophore and quencher, recovering the quenched fluorescence of Mca. Fluorescence increase is directly related to MMP activities and is monitored at Ex/Em=325 nm/393 nm (10, 15-20).

Cat#	Product Name	Sequence	Cleaved by
27076, 27077	390 MMP FRET Substrate I	Mca-PLGL-Dap(Dnp)-AR-NH_2	MMP-7/8/9/13
27078, 27079	390 MMP FRET Substrate II	Mca-PLGL-Dap(Dnp)-AR	N.D.*
27090, 27091	390 MMP FRET Substrate III	Mca-PLA-Nva-Dap(Dnp)-AR-NH_2	MMP-7/8/9/13
27108, 27109	390 MMP FRET Substrate IV	Mca-P-Cha-G-Nva-HA-Dap(Dnp)-NH_2	MMP-8/9/13
62027	390 MMP FRET Substrate V	Mca-PLGLEEA-Dap(Dnp)-NH_2	MMP-12
27110, 27111	NFF-2	Mca-RPKPYA-Nva-WM-K(Dnp)-NH_2	MMP-2/3/8/9/12
27114, 27115	NFF-3	Mca-RPKPVE-Nva-WR-K(Dnp)-NH_2	MMP-3
27159, 27160	Mca MMP FRET Peptide Fluorescence Standard I	Mca-PL	N/A
27112, 27113	Mca MMP FRET Peptide Fluorescence Standard II	Mca-RPKPQ	N/A

490 MMP FRET Substrates

In this category, all except one substrate uses EDANS/DABCYL as the FRET pair. The one exception uses an NBD/DMC pair. In the intact substrate, DABCYL quenches the fluorescence of EDANS. Proteolytic cleavage of the FRET peptide separates the fluorophore and quencher, recovering the quenched fluorescence of EDANS. Fluorescence increase is directly related to MMP activities and is monitored at Ex/Em=340 nm/490 nm.

Cat#	Product Name	Sequence	Cleaved by
25350, 25350-5	TNO211	DABCYL-γ-Abu -PQGL-E(EDANS)-AK-NH_2	MMP-2/8/12/13/14
27061, 27062	TNO113	DABCYL-γ-Abu-P-Cha-Abu-Smc-HA-E(EDANS)-AK-NH_2	MMP-8/13
27104, 27105	490 MMP FRET Substrate III	DABCYL-RPLALWRS-EDANS	MMP-7
60637	490 MMP FRET Substrate IV	DabcylPlus™-KPLA-Nva-D(Edans)-AR-NH_2	MMP-1/2/3/8/9/12/13
60563-1	490 MMP FRET Substrate V	NBD-RPKPLA-Nva-WK(DMC)-NH_2	MMP-1/7/12/13
60552-1	490 MMP FRET Substrate VI	Dabcyl Plus-KPLA-Nva-Dap(AMCA)AR-NH_2	N.D.*
60553-1	490 MMP FRET Substrate VII	Dabcyl-KPLA-Nva-Dap(AMCA-X)-NH_2	N.D.*

*Not determined

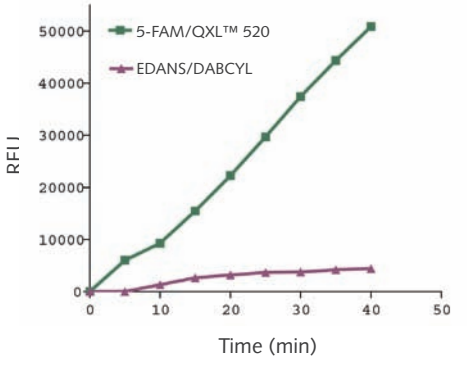

Figure 8. An MMP generic FRET substrate containing 5-FAM/QXL™ 520 shows higher sensitivity than the EDANS/DABCYL based FRET substrate.

520 MMP FRET Substrates

A novel FRET pair, 5-FAM/QXL™ 520 has been designed by AnaSpec to facilitate the assay of MMPs. 5-FAM has excellent extinction coefficient (79,000$M^{-1}cm^{-1}$), high quantum yield (0.92), and excitation and emission wavelengths at 490 nm/520 nm. QXL™ 520 has an absorption spectrum which completely overlaps the emission spectrum of 5-FAM (Figure 4, p.4), thus making it an ideal quencher for 5-FAM. It also has good water solubility. The performance of 5-FAM/QXL™ 520 FRET peptide outshines other FRET substrates, such as Trp/Dnp, Mca/Dnp, and EDANS/DABCYL. Substrates containing this novel FRET pair provide better enzyme kinetics, higher sensitivity, and show less interference from autofluorescence of cellular components and test compounds. In the intact peptide substrate, the fluorescence of FAM (donor) is quenched by QXL™ 520 ("dark" quencher). Proteolytic cleavage of the FRET peptide separates the donor and the quencher, recovering the quenched fluorescence of 5-FAM that is directly related to the MMP activities and can be monitored at Ex/Em = 490 nm/520 nm.

Cat#	Product Name	Sequence	Cleaved by
60568-01	520 MMP FRET Substrate I	QXL™ 520-PLGLWAr-K(5-FAM)-NH_2	MMP-13
60569-01	520 MMP FRET Substrate II	QXL™ 520-PLALWAR-K(5-FAM)-NH_2	MMP-1/7/8/11/12/13
60570-01	520 MMP FRET Substrate III	QXL™ 520-PLG-C(Me)-HAr-K(5-FAM)-NH_2	MMP-1/2/8/9/12/13/14
60571-01	520 MMP FRET Substrate IV	5-FAM-PLA-Nva-Dap(QXL520™)-AR-NH_2	MMP-1/2/7/8/12/13
60572-01	520 MMP FRET Substrate V	5-FAM-PLGL-Dap(QXL520™)-AR-NH_2	MMP-1/2/7/8/12/13
60573-01	520 MMP FRET Substrate VI	QXL™ 520-PLGMWSR-K(5-FAM)-NH_2	MMP-2/13
60574-01	520 MMP FRET Substrate VII	QXL™ 520-PYAYWMR-K(5-FAM)-NH_2	MMP-7/12/13
60575-01	520 MMP FRET Substrate VIII	QXL™ 520-RPKPLA-Nva-W-K(5-FAM)-NH_2	MMP-7/12/13
60576-01	520 MMP FRET Substrate IX	QXL™ 520-RPLALWR-K(5-FAM)-NH_2	MMP-1/2/7/8/12/13
60577-01	520 MMP FRET Substrate X	QXL™ 520-PLAYWAR-K(5-FAM)-NH_2	MMP-13
60578-01	520 MMP FRET Substrate XI	5-FAM-P-Cha-G-Nva-HA-Dap(QXL™ 520)-NH_2	MMP-1/2/8/12/13
60579-01	520 MMP FRET Substrate XII	5-FAM-RPKPYA-Nva-WM-K(QXL™ 520)-NH_2	MMP-1/2/3/12/13
60580-01	520 MMP FRET Substrate XIII	5-FAM-RPKPVE-Nva-WR-K(QXL™ 520)-NH_2	MMP-3/12
60581-01	520 MMP FRET Substrate XIV	QXL™ 520-γ-Abu-P-Cha-Abu-Smc-HA-Dab(5-FAM)-AK-NH_2 (Smc=S-Methyl-L-cysteine)	MMP-1/2/3/7/8/9/10/12/13/14
60582-01	520 MMP FRET Substrate XV	QXL™ 520-γ-Abu-PQGL-Dab(5-FAM)-AK-NH_2	MMP-1/2/7/8/12/13/14
60583-01	520 MMP FRET Substrate XVI	QXL™ 520-RPKPQQFW-K((5-FAM)-NH_2	MMP-12/13
60554-1	520 MMP FRET Substrate XVII	QXL™ 520-KPLA-Nva-Dap(5-FAM)-AR-NH_2	N.D.*
60584-01	5-FAM MMP FRET Peptide Fluorescence Std I	5-FAM-PL	N/A

*Not determined

580 MMP FRET Substrate

With higher emission wavelength, this substrate is ideal for use in experiments where the test compounds might have strong autofluorescence at shorter wavelength. Detection of this substrate is at Ex/Em = 547 nm/574 nm.

Cat#	Product Name	Sequence	Cleaved by
60585-01	580 MMP FRET Substrate I	QXL™ 570-KPLA-Nva-Dap(5-TAMRA)-AR-NH_2	MMP-1/2/8/9/13

Related Products

MMP Biotinylated Substrate I, Biotin-LC-PLGLRAY-NH_2 - cat# 27080 (1 mg), 27081 (5 mg).
MMP Colorimetric Substrate I, Ac-PLG-SCH[$CH_2CH(CH_3)_2$]-CO-LG-OC_2H_5 (Abs=412 nm) - cat# 27096 (1 mg), 27097 (5 mg).
MMP HPLC Substrate I, Dnp-PLG-C(Me)-HAr-NH_2 (Abs=360 nm) - cat# 27084 (1 mg), 27085 (5 mg).
MMP HPLC Substrate III, Dnp-P-Cha-G-C(Me)-HAK[Abz(N-Me)]-NH_2 (Abs=360 nm) - cat# 27088 (1 mg), 27089 (5 mg).
MMP Peptide Inhibitor I, Ac-RCGVPD-NH_2 - cat# 27157 (1 mg), 27158 (5 mg).
CTT, Gelatinase Inhibitor, CTTHWGFTLC (Disulfide Bridge: 1-10) - cat# 62054 (1 mg).
Anti-MMPs, include *new* zebrafish specific MMP antibodies: Search online by typing "anti-MMP" in the search box.

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1. Birkedal-Hansen, H. et al. *Crit. Rev. Oral Biol. Med.* **4**, 197 (1993).
2. Lenhart, J. et al. *Endocrinol.* **142**, 3941 (2001).
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Renin-Angiotensin System - Renin Assay Kits

The renin–angiotensin system (RAS) plays a central role in the regulation of blood pressure and electrolyte homeostasis (1). At the first and rate-limiting step of the RAS cascade, renin (EC 3.4.23.15), a highly specific aspartyl protease, cleaves angiotensinogen, produced in the liver, to yield angiotensin I, which is further converted into angiotensin II by Angiotensin Converting Enzyme (ACE). Angiotensin II constricts blood vessels leading to increased blood pressure. It also increases the secretion of ADH and aldosterone, and stimulates the hypothalamus to activate the thirst reflex. Since an overactive renin-angiotensin system leads to hypertension, renin is an attractive target for the treatment of this disease. In the past decade, a considerable number of structurally different synthetic renin inhibitors of excellent (sub-nanomolar) potency and selectivity have been described (2-4); however, in order to accelerate the drug discovery process and to perform automated inhibitor screening, a continuous, homogeneous assay with femtomolar sensitivity range is needed.

The SensoLyte™ Renin Assay Kits provide convenient assays for HTS of renin inhibitors and for continuous assay of renin activity. A 5-FAM/QXL™ 520 FRET peptide is used in the SensoLyte™ 520 Renin Assay Kit and an Amp/Dnp FRET peptide in the SensoLyte™ 390 Renin Assay Kit. The general sequence of these FRET peptides is derived from the cleavage site of renin (5). In the intact FRET peptides, the fluorescence of 5-FAM and Amp is quenched by QXL™ 520 and Dnp, respectively. Upon cleavage into two separate fragments by renin, the fluorescence of 5-FAM or Amp is recovered, and monitored at their respective excitation/emission wavelengths. With a high fluorescence quantum yield and long emission wavelength, the signal of 5-FAM can be detected with less interference from autofluorescence of cellular components and test compounds. Compared to an EDANS/DABCYL FRET substrate, the 5-FAM/QXL™ 520 FRET substrate is about 40 fold more sensitive and can detect 0.8 ng/ml of renin. Table 6 shows a comparison of the two assay kits, as well as an assay kit from another company.

Table 6. Comparison data of the two SensoLyte™ Renin Assay kits and a kit from Company B.

	SensoLyte™ 520 Renin Assay Kit Cat# 72040	SensoLyte™ 390 Renin Assay Kit Cat# 72039	Company B
FRET pair	5-FAM/QXL™ 520	Amp/Dnp	EDANS/DABCYL
Ex/Em (nm/nm)	490/520	330/390	335/495
Min. Detectable Renin (ng/ml)	0.8	8	32
Interference from Autofluorescence	Minimal	Moderate	Moderate
Sensitivity	Ultra-sensitive	Good	Minimal

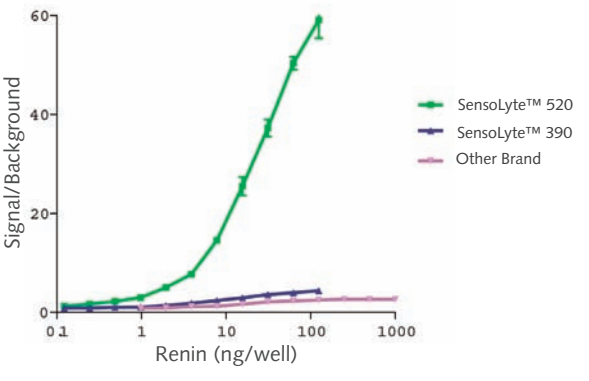

Figure 9. Comparison of three renin assay kits.

Related Products

Human Renin 5-FAM/QXL™ 520 FRET Substrate (Ex/Em=490 nm/520 nm) - cat# 61872 (1 mg), 61872-01 (0.1 mg).
Rat Renin 5-FAM/QXL™ 520 FRET Substrate (Ex/Em=490 nm/520 nm) - cat# 62334 (1 mg), 62334-01 (0.1 mg).
Renin 390 FRET Substrate I, R-E(EDANS)-IHPFHLVIHT-K(DABCYL)-R (Ex/Em=340 nm/490 nm) - cat# 62022 (1 mg).
Recombinant Renin, human - cat# 72041 (5 ug).
Rat Renin Inhibitor Peptide, WFML, Ac-HPFV-(Sta)-LF-NH_2 - cat# 60463-1 (1 mg).
Renin Inhibitor III, RRPFH-Sta-IHK-NH_2, cat# 72065 (1 mg) - cat# 72066 (5 mg).
Angiotensin (2-9) FRET peptide, DABCYL-RVYIHPFH-EDANS - cat# 61231 (1 mg).
Angiotensins and related peptides. Search online under Peptides>Catalog Peptides>Angiotensins & Related Peptides.

References:

1. He, FJ. and GA. MacGregor, *J. Renin Angiotensin Aldosterone Syst.* **4**, 11 (2003).
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Renin-Angiotensin System - ACE2 Assay Kit

Angiotensin I converting enzyme 2 (ACE2), the newest member of the renin angiotensin system (RAS), is a zinc metallopeptidase that plays a central role in the control of angiotensin peptides (1, 2). ACE2 has direct effects on cardiac function (3), and is expressed predominantly in vascular endothelial cells of the heart and the kidneys (2). ACE2 may also protect kidneys in early stages of diabetes (4). In addition to its role in the regulation of hypertension, ACE2 is a functional receptor for the coronavirus that causes severe acute respiratory syndrome, SARS (5). ACE2 is considered an important therapeutic target for controlling cardiovascular diseases, kidney disease and SARS outbreaks.

The SensoLyte™ 390 ACE2 Activity Assay Kit provides a convenient assay for HTS of ACE2 inhibitors and inducers and for continuous assay of ACE2 activity using an Mca/Dnp FRET peptide. In the FRET peptide, the fluorescence of Mca is quenched by Dnp. Upon cleavage into two separate fragments by the enzyme, the fluorescence of Mca is recovered and monitored at Ex/Em = 330 nm/390 nm. These assays, performed in a convenient 96-well microplate format, can detect subnanogram level of ACE2 activity.

	SensoLyte™ 390 ACE2 Activity Assay Kit Cat# 72086
FRET pair	Mca/Dnp
Ex/Em (nm/nm)	330/390

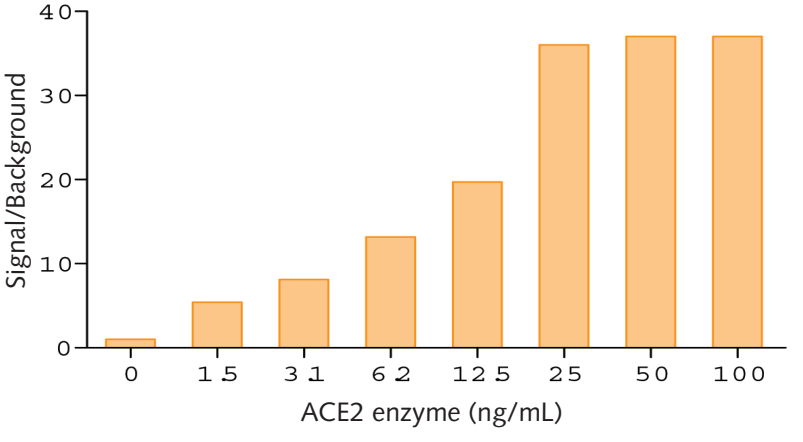

Figure 10. Sensitivity of Mca/Dnp-based ACE2 FRET substrate. Subnanogram range of ACE2 can be detected using this substrate. Serial dilutions of ACE2 protease were incubated with substrate at 37C for 1 h. End-point fluorescence signal was recorded at Ex/Em=330 nm/390 nm (FlexStation 384II, Molecular Devices).

Related Products

Angiotensins and related peptides. Search online under Peptides>Catalog Peptides>Angiotensins & Related Peptides.
SensoLyte™ 390 Renin Assay Kit (Ex/Em=330 nm/390 nm) - cat# 72039.
SensoLyte™ 520 Renin Assay Kit (Ex/Em=490 nm/520 nm) - cat# 72040.
DX600, ACE2 Specific Inhibitor, Ac-GDYSHCSPLRYYPWWKCTYPDPEGGG-NH_2 - cat# 62337 (0.1 mg).

References:
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α-Secretase or TACE Assay Kit

TACE (TNF-α converting enzyme), ADAM17 or α-secretase belongs to the ADAM (A Disintegrin and Metalloprotease) family of proteins, which are involved in myogenesis, neurogenesis, fertilization through the ectodomain shedding of cell surface proteins (1). TACE, the first 'sheddase' to be identified and the predominant protease responsible for the generation of soluble mature TNF-α (2), plays a crucial role in acute and chronic inflammation. Since TNF-α is a crucial mediator in the inflammatory process, considerable efforts have been made in the research and development of anti-TNF-α agents, for the purpose of reducing the severity of inflammatory responses in disease states (3, 4). The inhibition of TACE by a pharmacological agent may represent an alternative approach to modulate the effect of TNF-α (5). TACE is also responsible for the proteolytic cleavage of amyloid precursor protein, L-selectin and transforming growth factor-α (1, 6, 7).

The SensoLyte™ 520 TACE Activity Assay Kit is a homogeneous assay that can be used to detect the activity of TACE and for screening of TACE inhibitors. It contains a QXL™ 520/5-FAM FRET substrate, derived from a sequence surrounding the cleavage site of TACE (8). In the intact FRET peptide, the fluorescence of 5-FAM is quenched by QXL™ 520. Active TACE cleaves the FRET substrate into two separate fragments resulting in an increase of 5-FAM fluorescence which is monitored at Ex/Em=490 nm/520 nm. The long wavelength fluorescence of 5-FAM shows less interference from the autofluorescence of cellular components and test compounds.

	SensoLyte™ 520 TACE (α-Secretase) Activity Assay Kit Cat# 72085
FRET pair	QXL™ 520/5-FAM
Ex/Em (nm/nm)	490/520

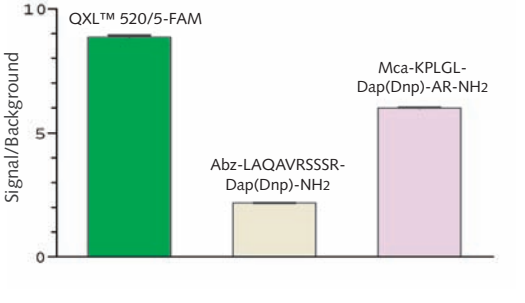

Figure 11. Comparison of three TACE assay kits. The SensoLyte™ 520 TACE Assay Kit contains the QXL™ 520/5-FAM FRET substrate, which is clearly superior to two FRET substrates used in other assay kits.

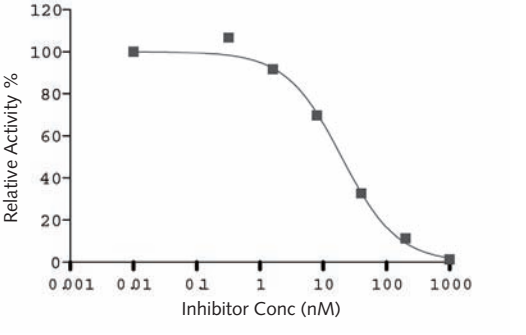

Figure 12. TAPI-0 inhibition of TACE activity measured with the SensoLyte™ 520 TACE Activity Assay Kit (TAPI-0 is a patented product of Research Corporation Technologies).

Related Products

α-Secretase Substrate, Mca-HQKLVFFAK(Dnp) (Ex/Em=325 nm/393 nm) - cat# 60271 (1 mg).

TACE FRET Substrate I, DABCYL-LAQAVRSSSR-EDANS (Ex/Em=335 nm/490 nm) - cat# 25043 (1mg), 25349 (5 mg).

Anti-TACE (CT) - cat# 28075.

Anti-Amyloids - cat# 53224, 54294-54295.

Anti-APPs - cat# 54095-54096.

Anti-Tau's - cat# 28017, 28018, 28023, 28024, 54960-54969, 54973-54979.

References:

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β-Secretase Assay Kit

Amyloid Precursor Protein, APP, a protein of about 770 amino acids, is cleaved by α-Secretase and β-Secretase. β-Secretase is also known as BACE1 (β-secretase APP cleaving enzyme) or memapsin. α-Secretase processes the majority of APP, producing a 83-amino acid C-terminal fragment, C83; while only a small amount is processed by β-Secretase, producing a 99-amino acid C-terminal fragment, C99. Subsequent cleavages of C83 and C99 by γ-Secretase produces a 3-kD (p3) protein in the former and a 4-kD (β-amyloid) protein in the latter and a C-terminal that is 57-59 residues long in both fragments (1-2). The 4-Kd consists of Aβ which are 39 to 42 amino acids in length, with Aβ 1-42 being the major component of amyloid plaques which accumulates in the neurons of Alzheimer's disease (AD) brain (3, 4). Thus, β-secretase is an important target for developing drugs for AD.

The SensoLyte™ 520 β-Secretase Assay Kit provides a convenient assay for HTS of β-secretase inhibitors and for the continuous quantification of β-secretase activity using a HiLyte Fluor™ 488/QXL™ 520 based FRET substrate. The sequence of this FRET peptide is derived from the β-secretase cleavage site on the Swedish APP mutation (5, 6). This mutation enhances the ability of β-secretase to process APP and results in an early onset of AD. In the FRET peptide, the fluorescence of HiLyte Fluor™ 488 is quenched by QXL™ 520 until this peptide is cleaved into two separate fragments by β-secretase at the Leu-Asp bond. Upon cleavage, the fluorescence of HiLyte Fluor™ 488 is recovered, and can be continuously monitored at Ex/Em = 488 nm/520 nm. Assays are performed in a convenient 96-well microplate format. 384-well or 1536-well format can also be used with minor modifications.

	SensoLyte™ 520 β-Secretase Assay Kit Cat# 71144
FRET pair	HiLyte Fluor™ 488/QXL™ 520
Ex/Em (nm/nm)	488/520

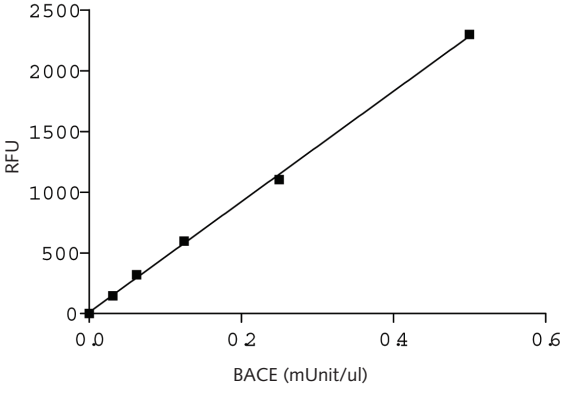

Figure 13. Sensitivity of the assay have been tested using serial dilution of BACE. FRET substrate was incubated with the indicated amount of BACE at 37C and fluorescence was measured after 40 min (FlexStation 384II, Molecular Devices). Sensitivity of the SensoLyte™ 520 β-Secretase assay was 0.03 mU/ul.

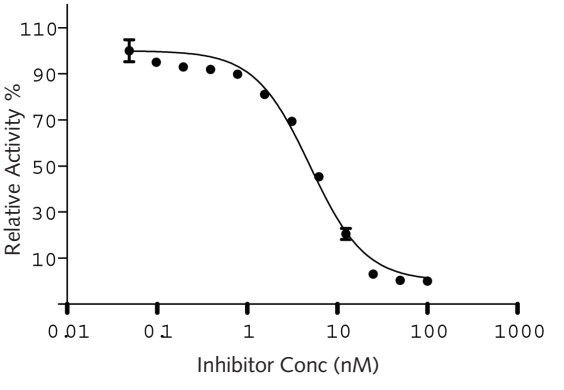

Figure 14. Inhibitor studies. To validate assay for inhibitor screening, FRET substrate (20 mM) was incubated with enzyme in the presence of a ß-secretase inhibitor (cat# 23960). Kinetic readings were taken every 5 min for 30 min at 37C (FlexStation 384II, Molecular Devices). The calculated IC_{50} was 5.62 nM.

Related Products

ß-Secretase Substrate 1a (control), Mca-EVKVDAEF-K(Dnp), Ex/Em=325 nm/393 nm - cat# 60268 (1 mg).

ß-Secretase Inhibitor 1, KTEEISEVN-Sta-VAEF (Sta = statine) - cat# 23958 (1 mg), cat# 23959 (5 mg).

ß-Secretase Inhibitor 2, KTEEISEVN-Sta-VAEF-NH_2 (Sta = statine) - cat# 23960 (1 mg), cat# 23961 (5 mg).

World's largest selection of β-amyloid peptides: Native or modified sequences, dye and/or biotin, heavy-isotope labeled, and much more. Search online under Peptide>Catalog Peptides>Amyloid Peptides.

Anti-Amyloids - cat# 53224, 54294-54295.

Anti-APPs - cat# 54095-54096.

Anti-Tau's - cat# 28017, 28018, 28023, 28024, 54960-54969, 54973-54979.

References:

1. Selkoe DJ. *Nature* **399**, A23 (1999).
2. Suh, Y-H. and F. Checler, *Pharmacol. Rev.* **54**, 469 (2002).
3. Suzuki, N. et al. *Science* **264**, 1336 (1994).
4. Iwatsubo, T. et al. *Neuron* **13**, 45 (1994).
5. Citron, M. et al. *Nature (London)* **360**, 672 (1992).
6. Mullan, M. et al. *Nat. Genet.* **1**, 345 (1992).

WNV (West Nile Virus) Protease Assay Kits

West Nile virus (WNV) is from the family *Flaviviridae* found in both tropical and temperate regions (1). The main route of human infection is through infected mosquito bites. Infection causes severe neurological disease and fatalities in both human and animal hosts. There is currently no effective vaccine or antiviral drug to protect against WNV infection (2). WNV contains a single-stranded, positive-sense RNA genome, which encodes three structural proteins: capsid, C; membrane M; envelope, E; and seven non-structural proteins: NS1, NS2A, NS2B, NS3, NS4A, NS4B, NS5 (3, 4). West Nile viral NS3 protease is absolutely essential (along with viral-encoded cofactor NS2B) for post-translational processing of a viral polypeptide precursor in infected host cells. This polypeptide provides the structural and functional viral proteins, and inhibition of its processing could represent a potential treatment for viral infections. Thus, this protease has become one of the key targets for developing anti-WNV drugs (5, 6).

The SensoLyte™ 570 West Nile Virus Protease Assay Kit provides a convenient, homogeneous assay for HTS of West Nile Virus NS3 protease inhibitors. It allows for continuous quantification of protease activity using a QXL™ 570/5-TAMRA FRET peptide. In the intact FRET peptide, the fluorescence of 5-TAMRA is quenched by QXL™ 570. Upon cleavage into two separate fragments by the WNV protease, the fluorescence of 5-TAMRA is recovered, and is monitored at Ex/Em = 540 nm/575 nm. The long wavelength fluorescence of 5-TAMRA shows less interference from autofluorescence of cellular components and test compounds.

	SensoLyte™ 570 West Nile Virus Protease Assay Kit Cat# 72080
FRET pair	QXL™ 570/5-TAMRA
Ex/Em (nm/nm)	540/575

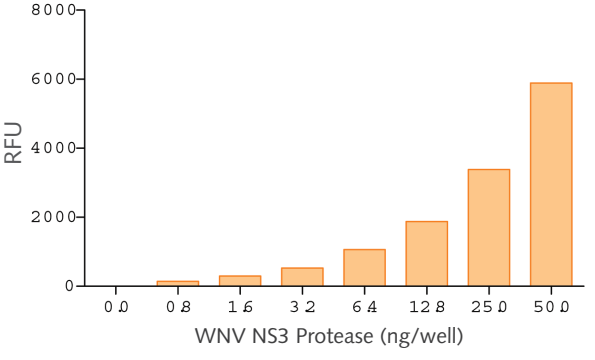

Figure 15. QXL™ 570/5-TAMRA FRET substrate (5 M) was incubated with serial dilutions of WNV NS3 protease at 37C for 1h. End-point fluorescence signal was recorded at Ex/Em=540 nm/575 nm (FlexStation 384II, Molecular Devices).

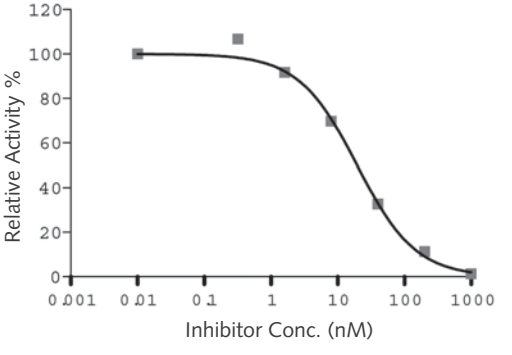

Figure 16. QXL™ 570/5-TAMRA FRET substrate (5 uM) was incubated with WNV NS3 protease (10 ng) in the presence of NS3 protease inhibitor (D-Arg)11-NH_2. Kinetic readings were taken at Ex/Em=540 nm/575 nm every 5 min for 60 min at 37C (FlexStation 384II, Molecular Devices). The calculated IC_{50} was 40.25 nM.

Shorter Wavelength Assay Kit

SensoLyte™ 440 West Nile Virus Protease Assay Kit, cat# 72079. This kit utilizes a fluorogenic peptide, Pyr-RTKR-AMC, which upon NS3 protease cleavage generates free AMC, emitting bright blue fluorescence, Ex/Em = 354 nm/442 nm.

Related Products

West Nile Virus NS3 Protease, recombinant - cat# 72081 (5 ug).
Antibodies against the different components of WNV - cat# 54198-54204, 54310.

References:

1. Shiryaev, SA. et al. *Biochem J.* **393**, 503 (2006).
2. Chappell, KJ. et al. *J. Biol. Chem.* **281**, 38448 (2006).
3. Hayes, CG., *Ann. NY. Acad. Sci.* **951**, 25 (2001).
4. van der Meulen, KM. et al. *Arch. Virol.* **150**, 637 (2005).
5. Brinton, MA. *Ann. Rev. Microbiol.* **56**, 371 (2002).
6. Lanciotti, RS. et al. *Science* **286**, 2333 (1999).
7. Mueller, NH. et al. *Int. J. Biochem. Cell Biol.* **39**, 606 (2007).

Green and Red Generic Protease Assay Kits

Protease assays are widely used in the investigation of protease inhibitors and also in the detection of protease activity in samples for quality inspection purpose (1-3). AnaSpec is pleased to provide two generic protease assay kits - The SensoLyte™ Green Protease Assay Kit and the SensoLyte™ Red Protease Assay Kit. These kits are optimized to detect activities of generic proteases, such as trypsin, chymotrypsin, thermolysin, proteinase K, protease XIV, and elastase. In both of these kits, casein, heavily labeled with pH-insensitive fluorophores, is used. It is labeled either with the green dye, HiLyte Fluor™ 488 or the red dye, 5(6)-TAMRA. These assays employ the same fluorophore for both the donor and the acceptor. The close proximity of the fluorophores to each other causes fluorescence quenching; however, upon proteolytic cleavage, there is release of a brightly green fluorescence for the SensoLyte™ Green Protease Assay Kit or red fluorescence for the SensoLyte™ Red Protease Assay Kit (Figure 17). The increase in fluorescence intensity is directly proportional to protease activity.

These kits do not require any separation steps and can be used to continuously measure kinetics of a variety of exopeptidases and endopeptidases in acidic or basic buffer since the two fluorophores are pH insensitive. Ample materials are provided to perform 500 assays in a 96-well format, with the protocol readily modified to run assays in a 384-well format.

	SensoLyte™ Green Protease Assay Kit Cat# 71124	SensoLyte™ Red Protease Assay Kit Cat# 71140
Fluorophore	HiLyte Fluor™ 488	5(6)-TAMRA
Ex/Em (nm/nm)	488/520	546/575

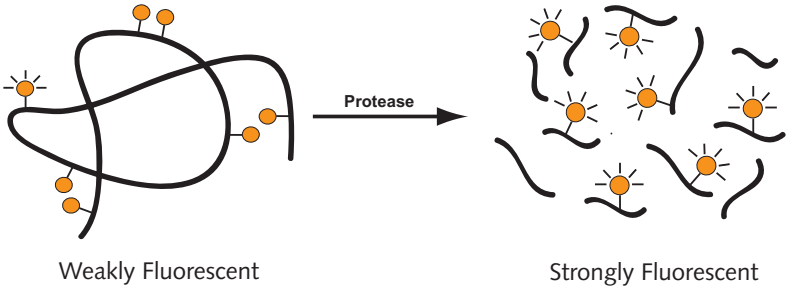

Figure 17. A schematic diagram of the quenched casein-fluorophore releasing fluorescence upon proteolytic cleavage.

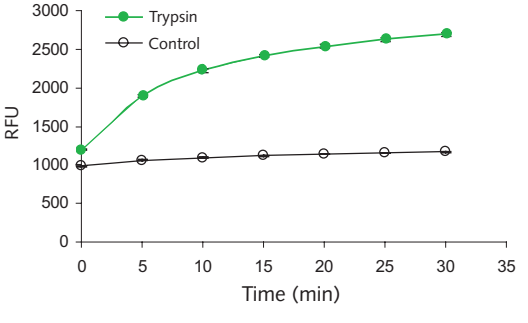

Figure 17. HiLyte Fluor™ 488-labeled casein was cleaved by 1 unit trypsin in assay buffer. The control wells contains HiLyte Fluor™ 488-labeled casein only, and no trypsin. Fluorescence was measured starting from Time 0, when trypsin was added, at Ex/Em=485±20 nm/528±20 nm (Flexstation 384II, Molecular Devices). Samples were done in duplicates.

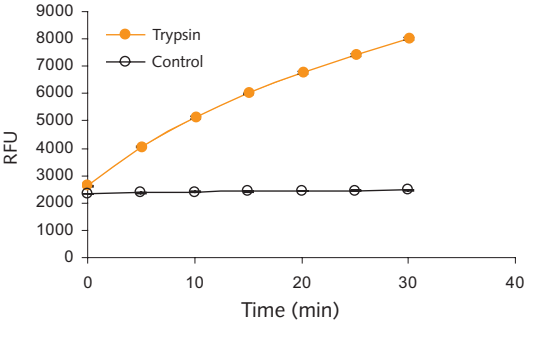

Figure 18. 5(6)-TAMRA labeled casein was cleaved by 1 unit trypsin in assay buffer. The control wells contain 5(6)-TAMRA-labeled casein only, and no trypsin. Fluorescence was measured starting from Time 0, when trypsin was added, at Ex/Em=530±25 nm/590±35 nm (Flexstation 384II, Molecular Devices). Samples were done in duplicates.

References:
1. Wiesner, R. and W. Troll, Anal. Biochem. 121, 290 (1982).
2. Sevier, ED., Anal.Biochem. 74, 592 (1976).
3. Spencer, PW. et al. Anal.Biochem. 64, 556 (1975).

Other FRET Substrates

Product	Sequence	Ex/Em (nm/nm)	Cat# (Size)
Aggrecanase (ADAM - TS - 4) Substrate, WAAG - 3R	Abz-TEGEARGSVI-Dap(Dnp)-KK-NH_2	340 / 420	60431-1 (1 mg)
Bacterial Sortase Substrate I	DABCYL-LPETG-EDANS	340 / 490	62231 (1 mg)
Caspase 1 (ICE) Substrate	DABCYL-YVADAPV-EDANS	340 / 490	60847 (1 mg)
Cathepsin D and E Substrate	Mca-GKPILFFRLK(Dnp)-r-NH_2	328 / 393	61793 (1 mg)
Cls Substrate, C2, Second Complement Component	5-FAM-SLGRKIQIQ-K(QXL™ 520)-NH_2	490 / 520	61314 (0.5 mg)
Cls Substrate, C2, Second Complement Component	2Abz-SLGRKIQI-K(Dnp)-NH_2	320 / 420	61315 (1 mg)
Cls Substrate, C2, Second Complement Component	DABCYL-SLGRKIQI-EDANS	340 / 490	61317 (1 mg)
Cls Substrate, C4, Fourth Complement Component	Abz-GLQRALEI-Lys(Dnp)-NH_2	320 / 420	61316 (1 mg)
CMV Protease Substrate I	DABCYL-RGVVNASSRLA-EDANS	340 / 490	22998 (1 mg)
Malaria Aspartyl Proteinase Substrate I	DABCYL-ER-Nle-FLSFP-EDANS	340 / 490	24480 (1 mg) 24481 (5 mg)
Hemoglobin 3037a, Malaria Substrate II	DABCYL-GABA-ERMFLSFP-EDANS	340 / 490	62014 (1 mg)
Hemoglobin 3037a, Malaria Substrate III	DABCYL-GABA-ALERMFLSFP-EDANS	340 / 490	62015 (1 mg)
Hemoglobin 2837b, Plasmepsin II Substrate	EDANS-CO-CH2-CH2-CO-ALERMFLSFP-Dap(DABCYL)OH	340 / 490	62050 (1 mg)
Interferon alpha A (101-110)	GVGVTETPLM-E(EDANS)-GVGVTETPLM-K(DABCYL)-K	340 / 490	62166 (1 mg)
Protein Tyrosine Phosphatase Substrate	Mca-EDAEpYAAK(DNP)R-NH_2	328 /395	60512-1 (1 mg) 60512-5 (5 mg)
SARS-CoV PLP2 Optimal Substrate (813 - 823)	Abz-FRLKGGAPIKGV-EDDnp	320 / 420	61619 (1 mg)

FRET Building Blocks

As one of the world's leading providers of dye-labeled and FRET peptides, AnaSpec is pleased to offer the following lab-proven collection of dye-labeled amino acids and resins. Already conjugated with fluorescent dyes or quencher molecules, these ready-to-use building blocks eliminate the need for additional coupling steps that can be tedious and technically challenging. Used routinely in AnaSpec's own San Jose, CA-based manufacturing facilities, these dye-labeled amino acids and resins offer lab-proven quality for your most critical peptide synthesis needs.

Amino Acids	Size	Cat#
Fmoc-Asp(EDANS)-OH N-α-Fmoc-L-aspartic acid-β-[2-(1-sulfonyl-5-naphthyl)-aminoethylamide]	0.1 g 1 g 5 g	23492-01 23492 23493
Boc-Asp(EDANS)-OH N-α-t-Boc-L-aspartic acid-β-[2-(1-sulfonyl-5-naphthyl)-aminoethylamide]	100 mg 1 g 5 g	23486-01 23486 23487
Fmoc-Glu(EDANS)-OH N-α-Fmoc-L-glutamic acid-γ-[2-(1-sulfonyl-5-naphthyl)-aminoethylamide]	0.1 g 1 g 5 g	23494-01 23494 23495
Boc-Glu(EDANS)-OH N-α-t -Boc-L-glutamic acid-γ-[2-(1-sulfonyl-5-naphthyl)-aminoethylamide]	0.1 g 1 g 5 g	23488-01 23488 23489
Fmoc-Lys(Boc-Abz)-OH N-α-Fmoc-(N-t-Boc-2-aminobenzoyl)-L-lysine	1 g 5 g	23719 23720
Fmoc-Lys(DABCYL)-OH N-α-Fmoc-N-ε-(4;4-dimethylazobenzene-4′carbonyl)-L-lysine	0.1 g 1 g 5 g	23496-01 23496 23497
Boc-Lys(DABCYL)-OH N-α-t-Boc-N-epsilon-(4;4-dimethylazobenzene-4′-carbonyl)-L-lysine	100 mg 1 g 5 g	23490-01 23490 23491

FRET Building Blocks (con't)

Amino Acids (con't)	Size	Cat#
Fmoc-Lys(Dansyl)-OH N-α-Fmoc-N-ε-[5-(dimethylamino)naphthalene-1-sulfonyl]-L-lysine	1 g	22504
Fmoc-Dap(Dnp)-OH Fmoc-(N-β-(2,4-dinitrophenyl))-L-α, β-diaminopropionic acid	1 g 5 g	23380 23381
Fmoc-Lys(Dnp)-OH Fmoc-N$^{\varepsilon}$-2,4-dinitrophenyl-L-lysine	0.5 g	61140-F05
Fmoc-Lys(5-FAM)-OH N-α-Fmoc-N-ε-(5-carboxyfluorescein)-L-lysine	0.5 g 1 g	28271 27273
Fmoc-Lys(Mca)-OH N-α-Fmoc-N-ε-[(7-methoxycoumarin-4-yl)acetyl-L–lysine	0.5 g 1 g	61925-05 61925-1

Resins	Size	Cat#
Fmoc-Dap(Dnp) Wang resin N-α-Fmoc-N-β-(2,4-dinitrophenyl)-L-2,3-diaminopropionic acid Wang resin	0.5 g 1 g	23854 23855
Fmoc-Lys(DABCYL) Wang resin N-α-Fmoc-N-ε-4-(4-dimethylaminophenylazo)benzoyl-L-lysine Wang resin	0.5 g 1 g	23858 23859
Fmoc-Lys(Dnp) Wang resin N-α-Fmoc-N-ε-(2,4-dinitrophenyl)-L-lysine Wang resin	0.5 g 1 g	23856 23857

FRET Donors and Quenchers - Chemical Reactivities and Spectral Properties

Table 7. Chemical reactivities and spectral properties of FRET building blocks.

Quencher (Acceptor)	λ_{max} (nm)	Amine-Reactive	Thiol-Reactive	Carbonyl-Reactive (Amine-Containing)	Recommended FRET Donor
Dnp	348	Dnp-X, acid; Dnp-X, SE	Dnp C2 maleimide	Dnp C2 amine	Trp, Abz, Abz(N-Me), Mca
DABCYL	428	DABCYL, acid; DABCYL, SE	DABCYL C2 maleimide	DABCYL C2 amine DABCYL hydrazide	EDANS, AMCA
DABCYL Plus™	437	DABCYL Plus™ acid; DABCYL Plus™, SE	DABCYL Plus™ C2 maleimide	DABCYL Plus™ C2 amine DABCYL Plus™ hydrazide	EDANS, AMCA
QXL™ 490	488	QXL™ 490, acid; QXL™ 490, SE	QXL™ 490 C2 maleimide	QXL™ 490 C2 amine QXL™ 490 hydrazide	EDANS, AMCA
QXL™ 520	508 & 530	QXL™ 520, acid; QXL™ 520, SE	QXL™ 520 C2 maleimide	QXL™ 520 C2 amine QXL™ 520 hydrazide	FAM, FITC, Rh6G HiLyte Fluor™ 488
QXL™ 570	578	QXL™ 570, acid; QXL™ 570, SE	QXL™ 570 C2 maleimide	QXL™ 570 C2 amine QXL™ 570 hydrazide	HiLytePlus™ 555, HiLyte Fluor™ 555, Cy3®, TAMRA, ROX, Alexa Fluor® 555
QXL™ 610	594 & 628	QXL™ 610, acid; QXL™ 610, SE	QXL™ 610 vinyl sulfone	QXL™ 610 C2 amine QXL™ 610 hydrazide	ROX, Texas Red®, HiLyte Fluor™ TR
QXL™ 670	668	QXL™ 670, acid; QXL™ 670, SE	QXL™ 670 C2 maleimide	QXL™ 670 C2 amine QXL™ 670 hydrazide	HiLytePlus™ 647, HiLyte Fluor™ 647, Cy5® Alexa Fluor® 647
QXL™ 680	679	QXL™ 680, acid; QXL™ 680, SE	QXL™ 680 C2 maleimide	QXL™ 680 C2 amine QXL™ 680 hydrazide	HiLytePlus™ 647, HiLyte Fluor™ 647, Cy5® Alexa Fluor® 647

Trademarks of other companies: Alexa Fluor®, Texas Red-Molecular Probes (Invitrogen); Cy® dyes-GE Healthcare.

Guidelines in Designing FRET Peptide Substrates

1. Choose a donor/acceptor pair where the absorption spectrum of the quencher overlaps with the emission spectrum of the donor (see Table 7, p. 17 for recommendations). We generally use a fluorescent donor and a non-fluorescent acceptor (quencher) to make protease peptide substrates (AnaSpec's QXL™ 520 has been proven to be an efficient quencher for FAM and HiLyte Fluor™ 488). The choice of donor/acceptor pair may be limited by the kind of fluorometer filter on hand.

2. Within the same peptide sequence, the donor and acceptor molecules must be in close proximity (typically 10-100 Å) in order to get good quenching. Once an active protease recognizes and cleaves the substrate into two separate fragments, the increase in the donor-acceptor distance causes FRET efficiency to decrease, resulting in the recovery of the donor's fluorescence. The time-dependent increase in fluorescence intensity is related to the extent of substrate hydrolysis.

3. Beside using the native sequence, sequences containing unnatural amino acid or modified bonds other than a regular amide bond can be used to increase efficiency of cleavage, to protect the peptide from degradation or to increase solubility. For example, Ac-DE-Dap(QXL™ 520)-EE-Abu-ψ[COO]AS-C(5-FAMsp)-NH_2, where an ester bond is used in place of an amide bond to increase cleavage efficiency.

4. Most fluorophores are amino reactive, which means they can be conjugated to the α-amino group or the ε-amino group of Lysine.

5. Thiol reactive dyes can be used to conjugate to Cys-containing peptides. This is an economical way to utilize the dyes since the peptides can be HPLC purified first before reacting with the dyes.

6. For hydrophobic sequences, Lysines or Arginines may be added to increase solubility. These amino acids must be added at the appropriate positions without adversely affecting the protease recognition site.

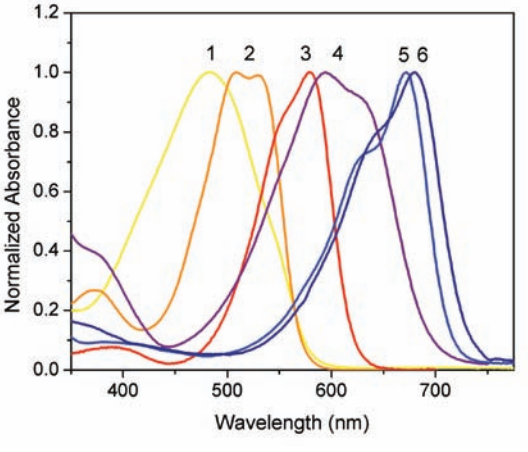

Figure 19. Normalized absorption spectra of QXL™ dark quenchers (free acids) in MeOH. 1). QXL™ 490; 2). QXL™ 520; 3). QXL™ 570; 4). QXL™ 610; 5). QXL™ 670; 6). QXL™ 680.