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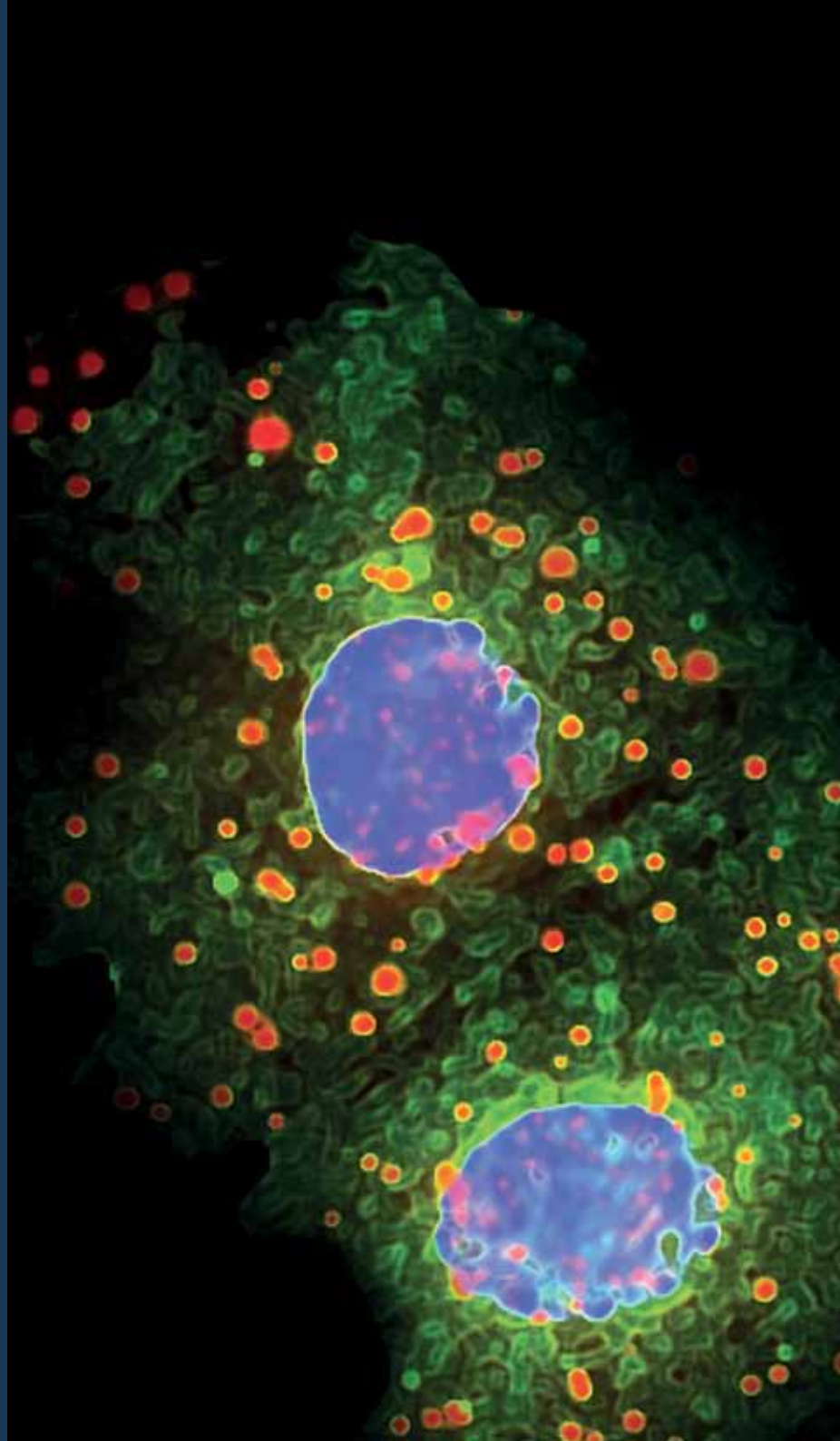
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SensoLyte[®] Assay kits

In recent years, FRET based assays have found broad applications, the major one being the detection of proteases activities. Proteases, also known as peptidases, are 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. 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 has spurred similar research for the research into other potential protease inhibitors and identification of protease involvement in diseases and their related cellular pathways:

- ▣ Apoptosis (Caspases)
- ▣ Bone disease (Aggrecanase)
- ▣ Cancer related proteases (MMPs)
- ▣ Neurodegenerative diseases (Secretase)
- ▣ Vascular diseases (Renin, Plasmin)
- ▣ Viral proteases

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. 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.

Eurogentec/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.

The SensoLyte[®] brand also comprises kits allowing the fluorometric and colorimetric detection of other enzymes implicated in

- ▣ Oxidative stress
- ▣ Epigenetics
- ▣ ELISA detection
- ▣ Gene expression

Measuring the activity of a protease with SensoLyte[®] Assay kits

- ▣ Proteases have low expression levels, highly sensitive detection needed
- ▣ Proteases are tightly controlled (expression & activity), the expression level of the protein is not sufficient in the study of a proteolysis process
- ▣ Protease inhibitors have proven their efficiency as drug, drug discovery needs high throughput screening (HTS) compatible methods

Catalogue Peptides GO[™] Peptides



All FRET Peptides substrates and enzymes inhibitors

But also :

- ▣ β -Amyloids Peptides
- ▣ MOG Peptides
- ▣ Phosphopeptides

High purity peptides, ready to be shipped

www.eurogentec.com

Alzheimer's disease



β -Amyloids ($A\beta$), the core components of neuritic plaques seen in Alzheimer's disease (AD) brains, are peptide of 39 to 42 amino acid in length. These are formed after sequential cleavage of the transmembrane Amyloid Precursor Protein (APP) by β and γ -secretase¹⁻³. β -Secretase is also known as BACE1 (β -secretase APP cleaving enzyme) or memapsin. In healthy brains APP is predominantly processed by α -Secretase (TACE), producing a 83-amino acid C-terminal fragment, C83.

Cathepsin D and Calpains (μ and m) are other proteases that can be related to Alzheimer's disease.

References

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2. Suh, Y-H. and F. Checler, *Pharmacol. Rev.* 54, 469 (2002)
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Results

Sensolyte[®] 520 TACE Assay kits sensitivity

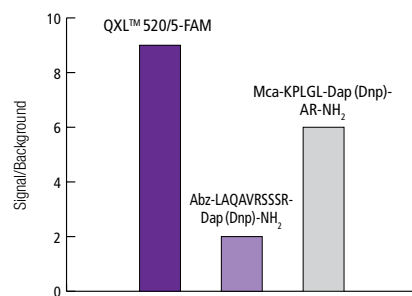


Figure 1: The Sensolyte[®] 520 TACE Assay kit contains the QXL™ 520/5-FAM FRET substrate, which is clearly superior to two FRET substrate used in other assay kits.

α - Secretase inhibitor studies

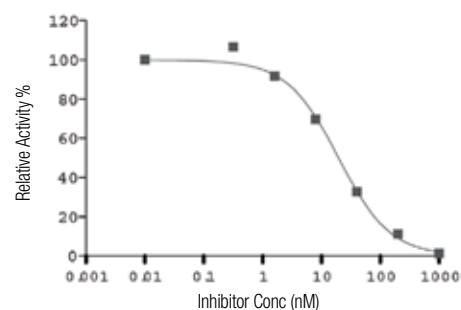


Figure 2: TAPI-O inhibition of TACE activity measured with Sensolyte[®] 520 TACE Activity Assay kit (TAPI-O is a patented product of Research Corporation Technologies).

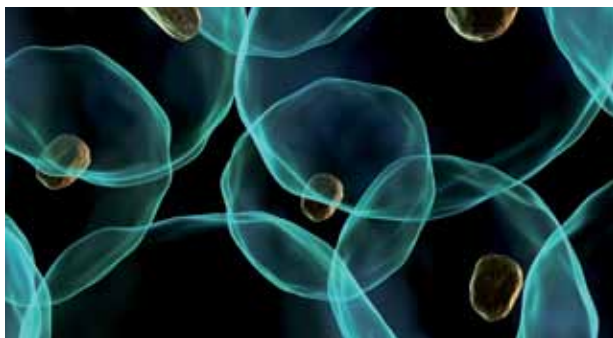
Assay kit overview

Enzyme/Antibody	Detection	Product Detection	Reference
α -secretase	Fluorimetric	Sensolyte [®] 520 TACE (a-Secretase) Activity Assay Kit	AS-72085
		Calpain	
Calpain	Fluorimetric	Sensolyte [®] 520 Calpain Activity Assay Kit	AS-72149
	Fluorimetric	Sensolyte [®] AMC Calpain Activity Assay Kit	AS-72150
		Cathepsin D	
Cathepsin D	Fluorimetric	Sensolyte [®] 520 Cathepsin D Assay Kit	AS-72097
	Fluorimetric	Sensolyte [®] 390 Cathepsin D Assay Kit	AS-72098

Product citations

- Sensolyte[®] 520 TACE Assay kit
Hurst, LA. *et al.* (2009) *J Neuroimmunol* 210, 108. Walker, EJ. and GA. Rosenberg (2009) *Exp Neuro* 216, 122. Cardellini, M. *et al.* (2009) *Diabetes* 58, 2396.
- Sensolyte[®] AMC Calpain Assay
Gallery, M. *et al.* (2007) *Mol Cancer Ther* 6, 262.

Oxidative stress



Thiol compounds, such as glutathione (GSH), cysteine, and homocysteine form a natural reservoir of the reductive capacity of a cell. They function as components of the intracellular and extracellular redox buffer and play important roles in a variety of biological processes, such as enzyme catalysis, redox-signaling protein folding, and free radical scavenging¹⁻³.

Reduced Glutathione (GSH) plays a crucial role in cellular defense against oxidative stress and functions as a cofactor for a variety of enzymes such as glutathione transferase and glutathione peroxidase. Upon oxidation, GSH is converted to GSSG. The concentrations of GSH and GSSG and their molar ratio are indicators of the cell's functionality and oxidative stress level⁴⁻⁵.

Reactive oxygen species (ROS) have an important role in a variety of biological events. Hydrogen peroxide (H_2O_2) is more stable than other ROS. It is often chosen to represent the ROS released by cell or cell organelles (e.g. mitochondria⁶, activated leukocytes⁷). H_2O_2 is also a coproduct of many oxidase-catalyzed reactions. Consequently, it can serve as an indicator of the activity of oxidases (e.g. NADPH oxidase⁸, glucose oxidase⁹, and monoamine oxidase¹⁰).

Results

Glutathione quantitation

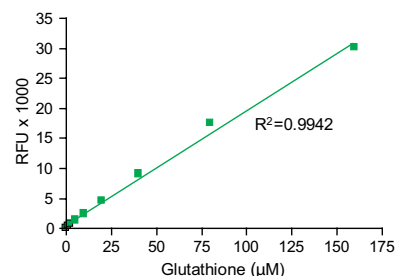


Figure 3: Detection of GSH with the Sensolyte®520 Thiol Quantitation Kit. Fluorescence signal was detected at Ex/Em=490/520 nm (FlexStation 384II).

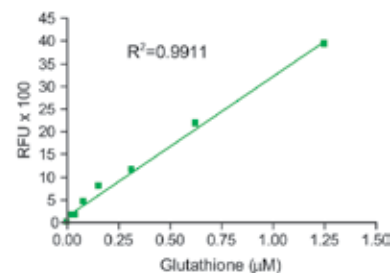


Figure 4: Expanded lower concentration range using the Sensolyte® 520 Thiol Quantitation Kit. Fluorescence signal was detected at Ex/Em=490/520nm (FlexStation 384II).

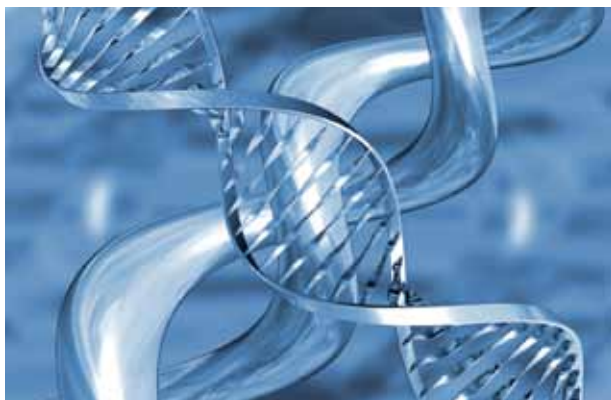
Assay kit overview

Enzyme/Antibody	Detection	Product Detection	Reference
Hydrogen Peroxyde	Fluorimetric	Sensolyte® ADHP	AS-71112
		Hydrogen Peroxide Assay Kit	
Reduced Glutathione (GSH)	Fluorimetric	Sensolyte® 520 Total GSH kit	AS-72154
	Colorimetric	Sensolyte® 520 Total GSH assay Kit	AS-72153
Thiol compounds	Colorimetric	Sensolyte® Thiol Quantitation Assay Kit	AS-72136
	Fluorimetric	Sensolyte® ABD-F Thiol Quantitation Assay Kit	AS-72137
	Fluorimetric	Sensolyte® 520 Thiol Quantitation Assay Kit	AS-72138

References

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- Winterbourn, CC. *et al. Free Radical Biol. Med.* 45, 549 (2008)
- Franco, R. *et al. Arch. Physiol. Biochem.* 113, 234 (2007)
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Epigenetics



Histone deacetylases (HDACs) catalyze the lysine amino acid deacetylation on histones leading to transcriptional gene repression. HDACs are divided into three classes. Class I (HDAC 1, 2, 3, 8) and class II (HDAC 4, 5, 6, 7, and 9) are zinc-containing hydrolase's enzymes¹⁻². The Sirtuin family of enzymes (Sir 1 to 7) forms the HDAC Class III and requires NAD as a cofactor³.

Histone demethylases such as LSD1 catalyse histones demethylation impacting gene transcription. LSD1 is histone H3 specific and can act either as a transcription co-repressor or co-activator⁴. The enzyme is implicated in cellular differentiation and has been considered as a potential target for drug discovery⁵.

References

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2. Verdin, E. *et al. Trends Genet.* 19, 286 (2003)
3. Blander, G. and L. Guarente *Annu. Rev. Biochem.* 73, 417 (2004)
4. Culhane, J. C. *et al. Curr. Opin. Chem. Biol.* 11, 561 (2007)
5. Shi, Y. *Nat. Rev. Genet.* 8, 829 (2007)

Results

HDAC kits sensitivity comparison

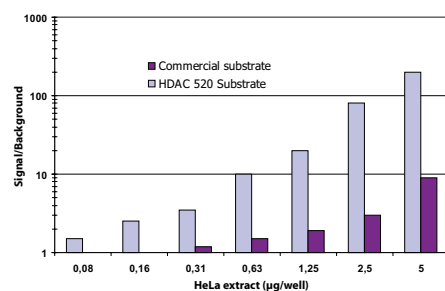


Figure 5: The Sensolyte[®] 520 HDAC substrate provides higher sensitivity and better linear range than an existing commercial substrate. HDAC substrates at a final concentration of 25 µM were incubated with HeLa nuclear extracts, followed by an additional 15 min incubation with a Trichostatin A-containing developer.

SIRT1 inhibitor screening

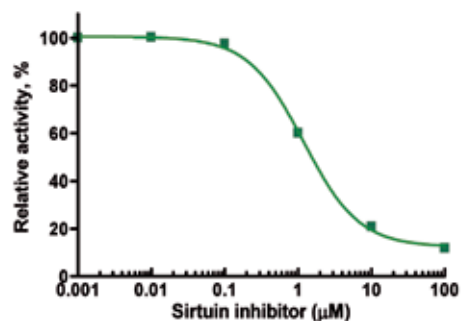
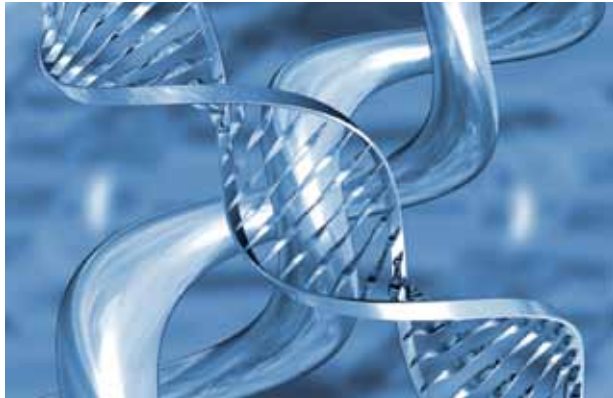


Figure 6: Inhibition of SIRT1 by Ro-31-8220 measured with the Sensolyte[®] 520 FRET SIRT1 Assay Kit.

Assay kit overview

Enzyme/ Antibody	Detection	Product Detection	Reference
Histone Deacetylase	Fluorimetric	Sensolyte [®] 520	
		HDAC Activity Assay Kit	AS-72084
	Fluorimetric	Sensolyte [®] 440	
		HDAC Activity Assay Kit	AS-72083
Sirtuin1 (Sirt1)	Fluorimetric	Sensolyte [®] 520 FRET Sirt1 Assay Kit	AS-72155
	Fluorimetric	Sensolyte [®] Green Sirt1 Assay Kit	AS-72156

Tissue Remodeling: MMPs



Matrix metalloproteinases (MMPs) are zinc-dependent endopeptidases. They are distinguished from other endopeptidases by their dependence on metal ions as cofactors, their ability to degrade extracellular matrix, and their specific evolutionary DNA sequence. These enzymes are responsible for the breakdown of connective tissues and are important in bone remodeling, the menstrual cycle and repair tissue damages.

MMPs are also thought to play a major role on cell behaviors such as cell proliferation, migration (adhesion/dispersion), differentiation, angiogenesis, apoptosis and host defense¹⁻².

References

1. Woessner, JF, Jr. and CJ. Taplin J. Biol. Chem. 263, 16918 (1988)
2. Woessner, JF, Jr. FASEB J. 5, 2145 (1991)

Results

Sensitivity of the SensoLyte® 520 MMPs Assay kit

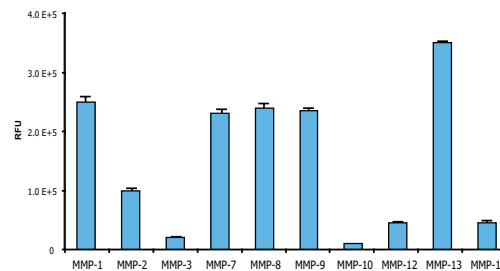


Figure 7: Detect the activity of MMPs using SensoLyte®520 generic MMP assay kit. The APMA-activated MMPs, 30ng each, were mixed with 5-FAM/QXL™ 520 FRET peptide substrate. The fluorescence signal was monitored with a fluorescence microplate reader (FlexStation 384II) at with the excitation at 490 nm and emission at 520 nm, cut off at 515 nm. Although different MMPs showed different cleavage rate on this FRET peptide, the FRET peptide can detect the activity of sub-nanogram of all MMPs (n=3, mean±S.D.)

The principle of SensoLyte® Plus 520 MMP-x assay kit

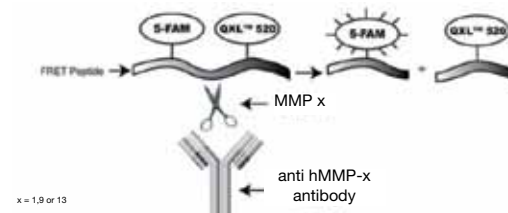
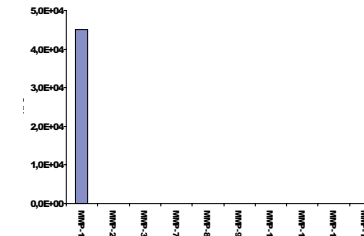
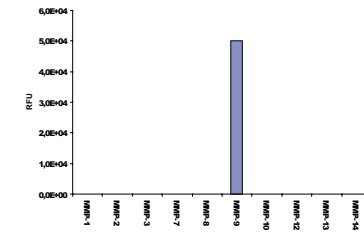


Figure 8: MMP-x (x=1,9 or 13) in biological samples is captured by immobilized MMP-x antibodies, and its proteolytic activity is measured by 5-FAM/QXL™520 FRET peptide. The fluorescence of 5-FAM (fluorophore) is quenched by QXL™520 (quencher) in the intact FRET peptide. Upon MMP-x cleavage, the fluorescence of 5-FAM is recovered and can be monitored at Ex/Em=490±20 nm/520± 20 nm.

The specificity of SensoLyte® Plus 520 MMP-1 assay kit



The specificity of SensoLyte® Plus 520 MMP-9 assay kit



The specificity of SensoLyte® Plus 520 MMP-13 assay kit

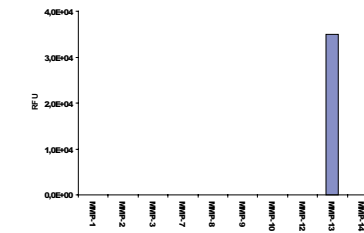


Figure 9: APMA-activated MMPs, 30 ng each, are added to the microplate pre-coated with anti-MMP-x antibody. After incubation, the plate was washed and the activity of MMPs detected by 5-FAM/QXL™520 FRET peptide substrate. 1h after adding the substrate, fluorescence signal was read (FlexStation 384II), at the excitation wavelength of 490 nm and emission wavelength of 520 nm, with cut off at 515 nm. The reading from all wells was subtracted with the reading from blank control, which contains FRET substrate but no MMPs. (n=3, mean±S.D.)

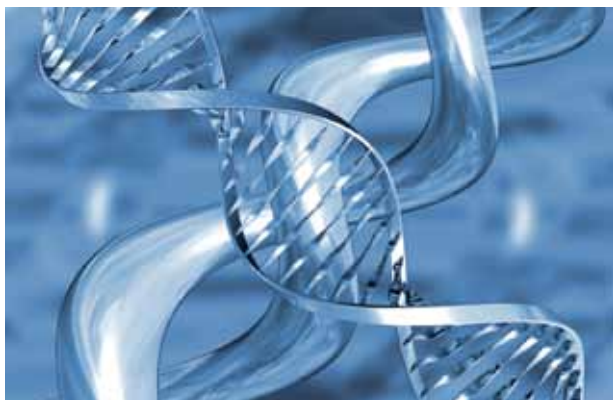
Assay kit overview			
Enzyme/ Antibody	Detection	Product Detection	Reference
MMP	Fluorimetric	SensoLyte® 570	AS-72101
		Generic MMP Assay Kit	
	Fluorimetric	SensoLyte® 520	AS-71158
		Generic MMP Activity Kit	
	Colorimetric	SensoLyte® Generic MMP Assay Kit	AS-72095
Fluorimetric	SensoLyte® 520 MMP Profiling Kit	AS-71136	
	Fluorimetric	SensoLyte® 520 MMP Substrate Sampler Kit	AS-71170
MMP-1	Fluorimetric and Enhanced Selectivity	SensoLyte® Plus 520 MMP-1 Assay Kit	AS-72012
		Fluorimetric	SensoLyte® 520 MMP-1 Assay Kit
	Fluorimetric	SensoLyte® 490 MMP-1 Assay Kit	AS-71128
	Colorimetric	SensoLyte® MMP-1 ELISA Kit	AS-72102
MMP-2	Fluorimetric	SensoLyte® 520 MMP-2 Assay Kit	AS-71151
		SensoLyte® 490 MMP-2 Assay Kit	AS-71129
	Colorimetric	SensoLyte® MMP-2 ELISA Kit	AS-72148
MMP-3	Fluorimetric	SensoLyte® 520 MMP-3 Assay Kit	AS-71152
		SensoLyte® 490 MMP-3 Assay Kit	AS-71130
	Colorimetric	SensoLyte® MMP-3 ELISA Kit	AS-72103
MMP-7	Fluorimetric	SensoLyte® 520 MMP-7 Assay Kit	AS-71153
	Fluorimetric	SensoLyte® 490 MMP-7 Assay Kit	AS-71132

Assay kit overview			
Enzyme/ Antibody	Detection	Product Detection	Reference
MMP-8	Fluorimetric	SensoLyte® 520 MMP-8 Assay Kit	AS-71154
	Fluorimetric	SensoLyte® 490 MMP-8 Assay Kit	AS-71133
	Colorimetric	SensoLyte® MMP-8 ELISA Kit	AS-72104
MMP-9	Fluorimetric and Enhanced Selectivity	SensoLyte® Plus 520 MMP-9 Assay Kit	AS-72017
		Fluorimetric	SensoLyte® 520 MMP-9 Assay Kit
	Fluorimetric	SensoLyte® 490 MMP-9 Assay Kit	AS-71134
	Colorimetric	SensoLyte® MMP-9 ELISA Kit	AS-72105
MMP-10	Fluorimetric	SensoLyte® 520 MMP-10 Assay Kit	AS-72024
	Colorimetric	SensoLyte® MMP-10 ELISA Kit	AS-72106
MMP-12	Fluorimetric	SensoLyte® 520 MMP-12 Assay Kit	AS-71157
	Fluorimetric	SensoLyte® 490 MMP-12 Assay Kit	AS-71137
MMP-13	Fluorimetric and Enhanced Selectivity	SensoLyte® Plus 520 MMP-13 Assay Kit	AS-72019
		Fluorimetric	SensoLyte® 520 MMP-13 Assay Kit
	Fluorimetric	SensoLyte® 490 MMP-13 Assay Kit	AS-71135
	Colorimetric	SensoLyte® MMP-13 ELISA Kit	AS-72107
MMP-14	Fluorimetric	SensoLyte® 520 MMP-14 Assay Kit	AS-72025

Product citations

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- SensoLyte® 520 MMP Substrate Sampler Kit Allington, TM. *et al.* (2009). *FASEB J* 23:4231-4243
- SensoLyte® PLUS 520 MMP-1 Assay Kit Cowan, RW. *et al.* (2009). *Bone* 44, 865.
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- SensoLyte® 520 MMP-2 Assay Kit Rahn, D. *et al.* (2009). *Am. J. Pathol.* 174, 206.

Apoptosis: Caspase



During apoptosis, caspases execute the disassembly of cellular components by proteolytic cleavage of a variety of substrates. Some of these caspases identify and cleave a specific peptide substrate, while others recognize the same peptide substrate². Both caspase-3 and caspase-7 have substrate selectivity for the amino acid sequence Asp-Glu-Val-Asp (DEVD).

Caspase-8, also known as FLICE, MACH and Mch5, is a key enzyme in the apoptosis pathway. Caspase-8 cleaves inactive pro-forms of effector caspase-3, -6 and -7, thereby activating them. These caspases are then capable of cleaving other cellular substrates such as PARP and DFF, which in turn induce apoptosis³⁻⁴.

References

- 1.Thornberry, NA. and Y. Lazebnik, *Science* 281, 1312-1316 (1998)
- 2.Villa, P. *et al. Trends Biochem. Sci.* 22, 388-393 (1997)
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Results

Dose-response curve of Camptothecin on caspase 3/7

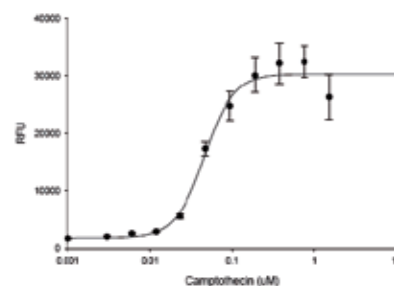


Figure 10: 1X10⁵/well Jurkat cells were treated with Camptothecin for 5 h. 50 µL/well of AMC caspase 3/7 substrate solution was added to apoptotic cells and incubated at room temperature for 30 min. The kinetic fluorescence signal was measured by a fluorescence microplate reader (FlexStation II384, Molecular Device, CA) with Ex/Em=354 nm/442 nm, cutoff 430 nm. EC₅₀= 0.026±0.003 µM.

Caspase-8 Detection

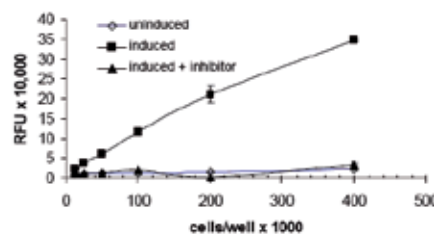


Figure 11: Jurkat cells were seeded to 96-well plates. Apoptosis was induced with 1 µg/mL staurosporine for 3 h. The identical population of cells after induction of apoptosis was incubated with caspase-8 specific inhibitor Ac-IETD-CHO at (25 µM final concentration). Fluorescence readings were taken 60 min. after addition caspase-8 substrate (Flexstation 384II, Molecular Devices). Each point represents the average of 3 replicates.

Assay kit overview

Enzyme/ Antibody	Detection	Product Detection	Reference
Caspase	Fluorimetric	SensoLyte® AFC Caspase Profiling Kit	AS-71116
	Fluorimetric	SensoLyte® AMC Caspase Profiling Kit	AS-71120
	Fluorimetric	SensoLyte® AFC Caspase Substrate Sampler Kit	AS-71117
	Fluorimetric	SensoLyte® AMC Caspase Substrate Sampler Kit	AS-71121
	Caspase-3/7	Fluorimetric	SensoLyte® Homogeneous AnaRed Caspase-3/7 Assay Kit
Fluorimetric		SensoLyte® Homogeneous Rh110 Caspase-3/7 Assay Kit	AS-71141
Fluorimetric		SensoLyte® Homogeneous AFC Caspase-3/7 Assay Kit	AS-71114
Fluorimetric		SensoLyte® Homogeneous AMC Caspase-3/7 Assay Kit	AS-71118
Caspase-8		Fluorimetric	SensoLyte® Homogeneous AFC Caspase - 8 Assay Kit
	Fluorimetric	SensoLyte® Homogeneous AFC Caspase - 8 Assay Kit	AS-72088-200

Product citations

- SensoLyte® Homogenous Rh110 Caspase-3/7 Assay Kit
Reynolds, AD. *et al.* (2009) *J. Immunol.* 182, 4137.
- Guo, ZM. *et al.* (2008). *Free Radical Bio. Med.* 44, 343
- Harris, GF. *et al.* (2008). *Arch. Otolaryngol. Head Neck Surg.* 134, 157
- SensoLyte® Homogenous AFC Caspase-3/7 Assay Kit
Ou, D. *et al. Human Immunol.* 66, 274 (2005).
- SensoLyte® Homogenous AMC Caspase-3/7 Assay Kit
Cheung, HC. *et al.* (2009). *Brain* 132, 2277.
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Bone-related proteolytic pathways & diseases



Cathepsin K is a cysteine protease involved in the degradation of bone matrix protein components during bone resorption. Produced by bone resorbing macrophages and synovial fibroblasts, cathepsin K cleaves proteins such as collagen type I, collagen type II and osteonectin. It has potential as a drug target in autoimmune diseases and osteoporosis. Aggrecanases are other bone related proteases. They belong to ADAMTS (A disintegrin and metalloprotease with thrombospondin motif) family of proteases. Aggrecanases cleave aggrecan, the major structural component of cartilage resulting in various symptoms of arthritis.

Bone development is also dependant on alkaline phosphatase activity. Changes in alkaline phosphatase level influence a variety of psychological and pathological events. Alkaline phosphatase is also widely used in ELISA for conjugation with secondary antibody and as a reporter for gene expression studies.

Results

Sensitivity of Sensolyte®520 Aggrecanase assay kit

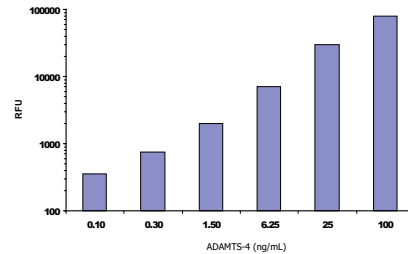


Figure 12: Fluorescence was measured after 1 hour incubation of FRET substrate with serial dilutions of Aggrecanase-1. Sensitivity of assay at these conditions was 0.1 ng/ml of enzyme.

Specificity of Sensolyte®520 Aggrecanase assay kit

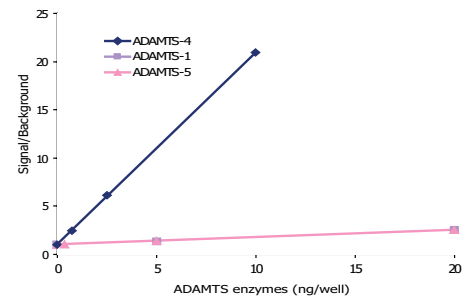


Figure 13: Various ADAMTS enzymes were incubated with ADAMTS-4 FRET substrate. We confirmed that 5-FAM/TAMRA ADAMTS-4 FRET substrate can discriminate between members of ADAMTS family.

Assay kit overview

Enzyme/Antibody	Detection	Product Detection	Reference
Cathepsin K	Fluorimetric	SensoLyte® Rh110 Cathepsin K Assay Kit	AS-72152
	Fluorimetric	SensoLyte® 520 Cathepsin K Assay Kit	AS-72151
Aggrecanase-1	Fluorimetric	SensoLyte® 520 Aggrecanase-1 Assay Kit	AS-72114
	Colorimetric	SensoLyte® MG Phosphate Assay Kit	AS-71103
Phosphatase	Colorimetric	SensoLyte® pNPP Protein Phosphatase Assay Kit	AS-71105
	Fluorimetric	SensoLyte® FDP Protein Phosphatase Assay Kit	AS-71100
Phosphatase	Fluorimetric	SensoLyte® MFP Protein Phosphatase Assay Kit	AS-71104

Product citations

- Sensolyte® pNPP Alkaline Phosphatase Assay Kit
Stanisic, V. *et al. J Biol Chem* 284, 16135 (2009).
Haraguchi, N. *et al. Ann Surg Oncol* 15, 2927 (2008).
- Sensolyte® pNPP Protein Phosphatase Assay Kit
Cho, Y. *et al. Am J Respir Cell Mol Biol* 39, 412 (2008).
Coynce, CB. *et al. EMBO* 26, 4016 (2007).
Sensolyte® FDP Alkaline Phosphatase Assay Kit
Arany, S. *et al. J Cell Biochem* 106, 539.

Vascular diseases



Hypertension is a chronic medical condition in which the blood pressure is elevated. The renin–angiotensin system plays a central role in the regulation of blood pressure and electrolyte homeostasis, making Renin an attractive target for the treatment of related diseases.

ACE2 (Angiotensin I converting enzyme 2) exopeptidase is also considered as an important therapeutic target not only for controlling cardiovascular diseases but also kidney disease and severe acute respiratory syndrome (SARS) outbreaks.

Coagulation (process by which blood forms clots) is an important part of hemostasis. Thrombin is a serine protease functioning as a main executioner of the coagulation cascade. It converts soluble plasma fibrinogen into an insoluble fibrin clot and promotes platelet aggregation. Thrombin also influences a number of normal and pathological processes, including inflammation, tissue repair, embryogenesis angiogenesis, and tumor invasion. Plasmin, a serine protease derived from the conversion of plasminogen in blood plasma, degrades fibrin in blood clots. Vessels obstruction can also be caused by atherosclerosis (artery wall thickens as the result of an accumulation of fatty materials such as cholesterol) implying a lysosomal cysteine protease called Cathepsin S. Cathepsin S is also involved in other pathologies including cancer, obesity, but its major role is the degradation of the invariant peptide chain associated with the major histocompatibility complex.

Results

Renin inhibitor screening

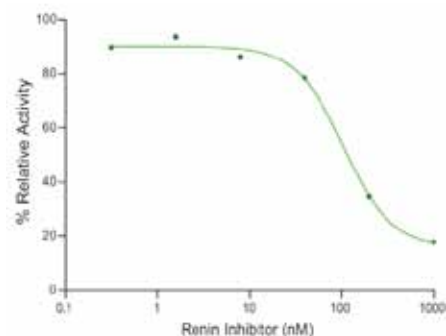


Figure 14: The inhibitory curve of renin inhibitor, Ac-HPFV-(Sta)-LF-NH2 using Sensolyte® 520 Rat Renin Assay Kit.

Sensolyte® Renin Assay kit sensitivity comparison

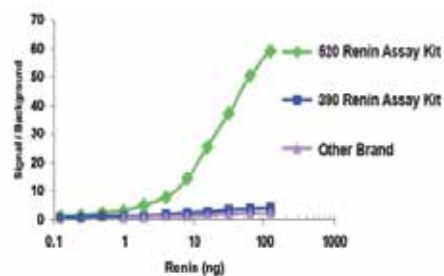


Figure 15: AnaSpec's new 520 Renin Inhibitor screening Assay Kit is the most sensitive renin assay kit offering 40 times greater sensitivity than the next leading brand.

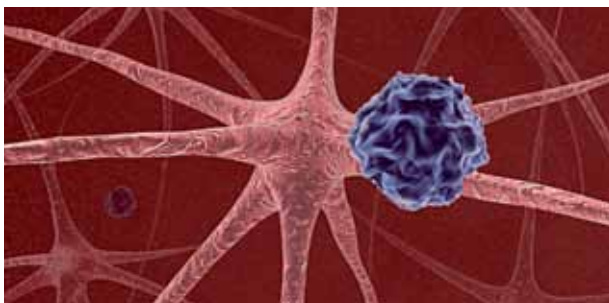
Assay kit overview

Enzyme/ Antibody	Detection	Product Detection	Reference
Thrombin	Fluorimetric	SensoLyte® 520 Thrombin Activity Assay Kit	AS-72129
	Fluorimetric	SensoLyte® AFC Thrombin Activity Assay Kit	AS-72130
Plasmin	Fluorimetric	SensoLyte® Rh110 Plasmin Activity Assay Kit	AS-72125
	Fluorimetric	SensoLyte® AFC Plasmin Activity Assay Kit	AS-72124
Cathepsin S	Fluorimetric	SensoLyte® 520 Cathepsin S Assay Kit	AS-72099
	Fluorimetric	SensoLyte® 440 Cathepsin S Assay Kit	AS-72100
Renin	Fluorimetric	SensoLyte® 520 Rat Renin Assay Kit	AS-72140
	Fluorimetric	SensoLyte® 520 Renin Assay Kit	AS-72040
	Fluorimetric	SensoLyte® 390 Renin Assay Kit	AS-72039
ACE2	Fluorimetric	SensoLyte® 390 ACE2 Activity Assay Kit	AS-72086

Product citations

- Sensolyte®520 Rat Renin Assay Kit
Du, D. *et al.* (2009). *Mol Biotech* 43, 1559. Živná, M. *et al.* (2009). *Am J Hum Genet* 85, 204.
- Sensolyte®390 ACE2 Activity Assay Kit
Cromlish, W. *et al.* *Basic & Clin. Pharmacol. Toxicol.* 182, 21 (2008).
Luhtala, S. *et al.* (2009). *J. Ocul. Pharmacol. Ther.* 25, 23.

Viral diseases



Viral proteases are regarded as important target for drug discovery against viral diseases. Effectively, they are important in the process of viral particle maturation by allowing cleavage of viral polyproteins.

In Human immunodeficiency virus-1 (HIV-1), the cleavage of the precursor polyproteins, Pr³⁹⁶ and Pr³⁹⁶ by aspartic protease is essential to give mature HIV infectious particles¹.

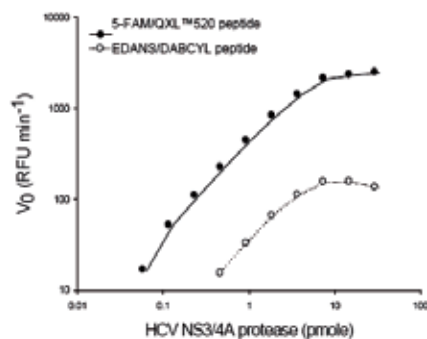
In hepatitis C virus (HCV), NS3/4A is required for the cleavage of viral nonstructural polyprotein at the NS3-NS4A, NS4A-NS4B, NS4B-NS5A, and NS5A-NS5B sites. For West Nile virus (WNV), the NS3 protease activity is absolutely essential to provide structural and functional viral proteins²⁻⁸.

References

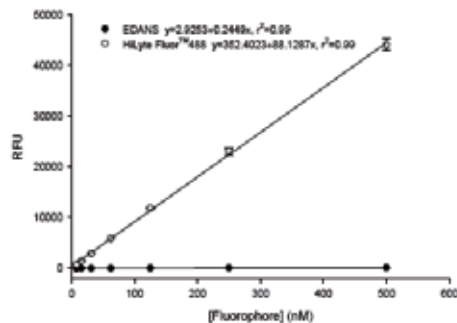
1. Seelmeier, S. *et al. Proc. Nat. Acad. Sci. U.S.A* 85, 6612 (1988)
2. Shiryayev, SA. *et al., Biochem J.* 393, 503 (2006)
3. Chappell, KJ. *et al., J. Biol. Chem.* 281, 38448 (2006)
4. Hayes, CG. *Ann. NY. Acad. Sci.* 951, 25 (2001)
5. Van der Meulen, KM *et al., Arch. Virol.* 150, 637 (2005)
6. Brinton, MA. *Annu. Rev. Microbiol.* 56, 371 (2002)
7. Lanciotti, RS. *et al., Science* 286, 2333 (1999)
8. Mueller, NH. *et al., Int. J. Biochem. Cell Biol.* 39, 606 (2007)

Results

Proteolytic cleavage of 5-FAM/QXL™520 and Edans/Dabcyl FRET peptide substrate by NS3/4A protease



Fluorescence intensity comparison of HiLyte Fluor™488 and EDANS



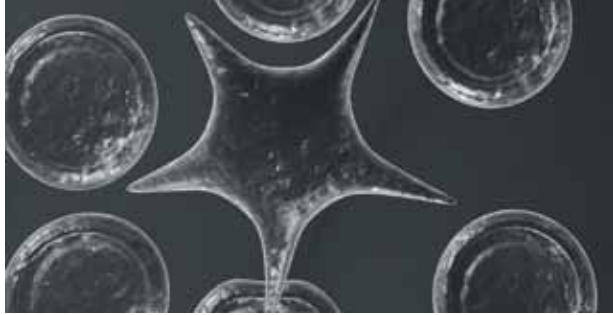
Assay kit overview

Enzyme/Antibody	Detection	Product Detection	Reference
HIV	Fluorimetric	SensoLyte® 620 HIV Protease Assay Kit	AS-71148
	Fluorimetric	SensoLyte® 520 HIV Protease Assay Kit	AS-71147
	Fluorimetric	SensoLyte® 490 HIV Protease Assay Kit	AS-71127
HCV	Fluorimetric	SensoLyte® 620 HCV Protease Assay Kit	AS-71146
	Fluorimetric	SensoLyte® 520 HCV Protease Assay Kit	AS-71145
	Fluorimetric	SensoLyte® 490 HCV Protease Assay Kit	AS-71126
	Fluorimetric	SensoLyte® 490 HCV Protease Assay Kit	AS-72087
WNV	Fluorimetric	SensoLyte® 570 West Nile Virus Protease Assay Kit	AS-72080
	Fluorimetric	SensoLyte® 440 West Nile Virus Protease Assay Kit	AS-72079

Product citations

SensoLyte® 520 HCV Assay Kit
Phuong, DT. *et al.* (2009). *Phytother. Res.* 23, 582.
Wang, S-Y. *et al.* (2009). *BBRC* 385, 230.
Ma, Y. *et al.* (2008). *J. Virol.* 82, 7624.

ELISA & Gene expression assays



Horseradish Peroxidase (HRP) or Alkaline Phosphatase (AP) protein conjugates are extensively used as secondary detection reagents in ELISA, immunohistochemistry and assay development. Some phosphatases serve as enzyme markers.

β -galactosidase (β -Gal), is one of the first and most popular reporter enzymes. β -galactosidase, encoded by the lacZ gene of *E. coli*, catalyzes the hydrolysis of β -galactosides into monosaccharides.

Reporter enzymes are widely used to study gene expression, protein-protein interactions¹ and normalization of transfection efficiency².

References

- Rossi, F. *et al.* PNAS 94, 8405 (1997)
- Thompson CD *et al.* Biotechniques 27, 824 (1999)

Results

ELISA using Alkaline phosphatase

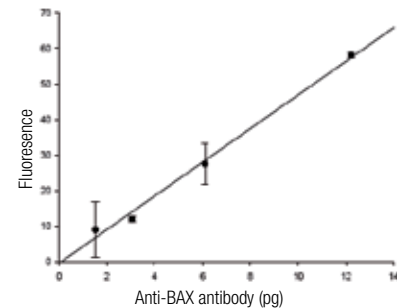


Figure 16: SensoLyte®FDP alkaline phosphatase ELISA assay kit was used to detect rabbit anti-BAX antibody. The assay can detect as low as 1 pg. The wells were coated with BAX-BSA. The fluorescence was read by a fluorescence microplate reader at Ex/Em=485±20 nm/528±20 nm.

β -galactosidase measurement

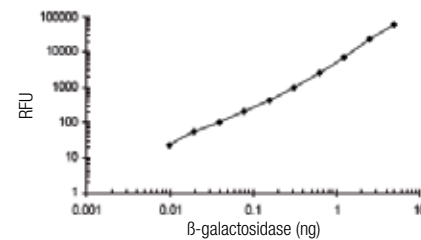


Figure 17: Detection of β -galactosidase with the SensoLyte® FDG β -galactosidase Assay Kit. Endpoint fluorescence signal was monitored at Ex/Em=490/520 nm (FlexStation 384II, Molecular Devices). The assay is able to detect as low as 10 pg of β -galactosidase (mean±S.D., n=3).

Assay kit overview

Enzyme/ Antibody	Detection	Product Detection	Reference
Alkaline Phosphatase	Colorimetric	SensoLyte® pNPP Alkaline Phosphatase Assay Kit	AS-72146
	Fluorimetric	SensoLyte® FDP Alkaline Phosphatase Assay Kit	AS-71109
	Luminometric	SensoLyte® Luminescent Alkaline Phosphatase Assay Kit	AS-72122
	Fluorimetric	SensoLyte® FDP Alkaline Phosphatase ELISA Assay Kit	AS-71101-M
	Fluorimetric	SensoLyte® FDP Alkaline Phosphatase ELISA Assay Kit	AS-71101-R
	Colorimetric	SensoLyte® pNPP Alkaline Phosphatase ELISA Assay Kit	AS-72147-G
	Colorimetric	SensoLyte® pNPP Alkaline Phosphatase ELISA Assay Kit	AS-72147-M
	Colorimetric	SensoLyte® pNPP Alkaline Phosphatase ELISA Assay Kit	AS-72147-R
	Luminometric	SensoLyte® Luminescent Alkaline Phosphatase ELISA Assay Kit	AS-72123
	Colorimetric	SensoLyte® pNPP Secreted Alkaline Phosphatase Reporter Gene Assay Kit	AS-72144

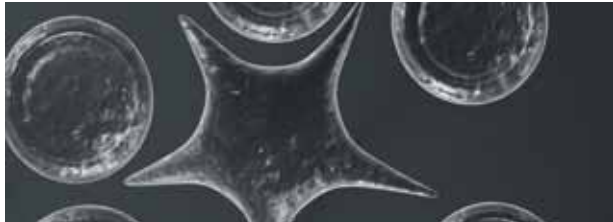
Assay kit overview

Enzyme/ Antibody	Detection	Product Detection	Reference
Alkaline Phosphatase	Fluorimetric	SensoLyte® FDP	AS-71108
		Secreted Alkaline Phosphatase Reporter Gene Assay Kit	
	Luminometric	SensoLyte®	
		Luminescent Secreted Alkaline Phosphatase Reporter Gene Assay Kit	
Peroxidase	Fluorimetric	SensoLyte® ADHP	AS-71110-M
		Peroxidase ELISA Assay Kit (mouse)	
	Fluorimetric	SensoLyte® ADHP	AS-71110-R
		Peroxidase ELISA Assay Kit (rat)	
	Luminometric	SensoLyte®	AS-72128
		Luminescent Peroxidase ELISA Assay Kit	
	Luminometric	SensoLyte®	AS-72127
		Luminescent Peroxidase Assay Kit	
	Fluorimetric	SensoLyte® ADHP	AS-71111
		Peroxidase Assay Kit	
β-galactosidase	Fluorimetric	SensoLyte® FDG	AS-72133
		b-Galactosidase Assay Kit	
	Fluorimetric	SensoLyte® MUG	AS-72132
		b-Galactosidase Assay Kit	
	Colorimetric	SensoLyte® ONPG	AS-72134
		b-Galactosidase Assay Kit	

Assay kit overview

Enzyme/ Antibody	Detection	Product Detection	Reference
Phosphatase	Colorimetric	SensoLyte® MG	AS-71103
		Phosphate Assay Kit	
	Colorimetric	SensoLyte® pNPP	AS-71105
		Protein Phosphatase Assay Kit	
	Fluorimetric	SensoLyte® FDP	AS-71100
		Protein Phosphatase Assay Kit	
Fluorimetric	SensoLyte® MFP	AS-71104	
	Protein Phosphatase Assay Kit		

Cell proliferation, viability and cytotoxicity assays



Lactate dehydrogenase (LDH) generally exists in prokaryotic, fungal and eukaryotic cells. The measurement of cytoplasmic LDH activity is a well-accepted assay to quantify viable cell numbers and monitor cell proliferation¹. On the other hand, the leakage of cytoplasmic LDH caused by the damage of cell membrane integrity is also a good indicator of cell death and is used to estimate cytotoxicity². The amount of living cells can also be measured by calcein fluorescence. Fluorescence Calcein is formed from Calcein acetoxymethyl ester (Calcein AM) hydrolysis by intracellular esterases in live cells³⁻⁴. The bright green fluorescence of calcein can be monitored at Ex/Em=494 nm /520 nm.

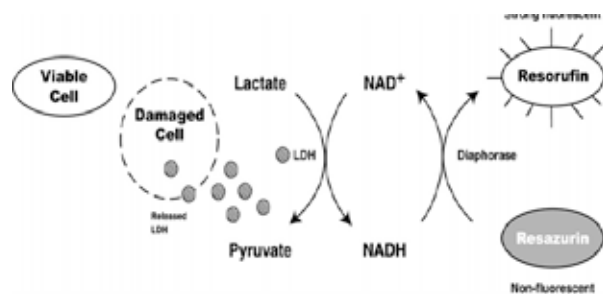


Figure 18: In a cell population of mixed viable cells and damaged cells, the DHL™ cell cytotoxicity assay kit only detects the dehydrogenases (e.g. LDH) activity released from damaged cell, and not those in live cells. In the enzyme-coupled reaction, dehydrogenases in the medium convert non-fluorescent resazurin to the strongly fluorescent resorufin, which can be monitored at Ex/Em= 530-560 nm/590 nm.

Results

Lactate Deshydrogenase measurement

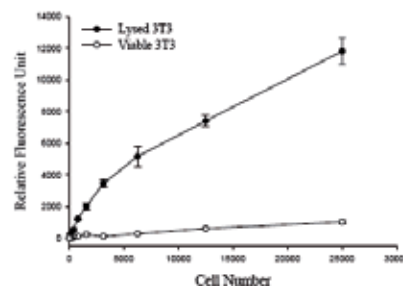


Figure 19: Lactate Deshydrogenase measurement with Sensolyte® cell cytotoxicity Assay Kit. Fluorescence signal was monitored at Ex/Em=530±30/590±30 nm. The assay can detect as few as 97 lysed 3T3 cells (>±3S.D.), living cells produce little fluorescence signal.

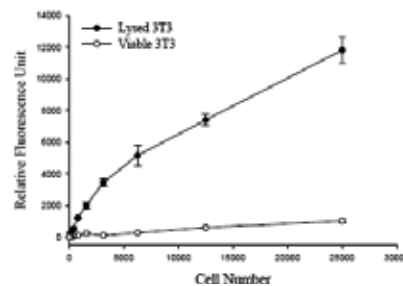


Figure 20: Lactate Deshydrogenase measurement by continuous monitoring of the change in resazurin fluorescence in living cells. Fluorescence was recorded at Ex/Em=530±30/590±30 nm up to 5 h. (mean±S.D., n=4 independent samples).

Assay kit overview

Enzyme/Antibody	Detection	Product Detection	Reference
Lactate dehydrogenase	Fluorimetric	DHL™ Cell Viability and Proliferation Assay Kit	AS-71300
	Fluorimetric	DHL™ Cell Viability and Proliferation Assay Kit	AS-71301
	Fluorimetric	DHL™ Cell Cytotoxicity Assay Kit	AS-71302
	Fluorimetric	DHL™ Cell Cytotoxicity Assay Kit	AS-71303
Calcein	Fluorimetric	Sensolyte® Calcein Cell Viability Assay Kit	AS-72126

Product citations

- DHL™ Cell Viability and Proliferation Assay Kit
Siegfried, J. M. *et al.* (2007) *Mol. Pharmacol.* Roelants, V. *et al.* (2008) *J. Nucl. Med.* 49, 1836.
Brennan, SE. *et al.* (2009) *Cancer Res.* 69, 5168.
- DHL™ Cell Cytotoxicity Assay Kit
Armant, DR. *et al.* *Development* 133, 751 (2006). Wolff, GS. *et al.* *Biol. Reprod.* 77, 53 (2007).
Leach, RE. *et al.* *Am. J. Obs. Gyne.* 198, 471e1 (2008).

Generic Protease Assay kits

The Sensolyte® Green Protease Assay Kit uses casein that is heavily labeled with HiLyte Fluor™ 488, a pH-insensitive green fluorophore, resulting in almost total quenching of its fluorescence. Proteolytic cleavage of this quenched casein-HiLyte Fluor™ 488 conjugate yields brightly green fluorescence, which can be continuously monitored at excitation/emission=488 nm/520 nm. The increase in fluorescence intensity is directly proportional to protease activity. This kit does not require any separation steps and can be used to continuously measure the kinetics of a variety of exopeptidases and endopeptidases in acidic and basic buffer.

The Sensolyte® Red Protease Assay Kit uses casein heavily labeled with the red fluorophore 5(6)-TAMRA, giving upon cleavage a bright red fluorescence which can be continuously monitored at excitation/emission=546 nm/575 nm.

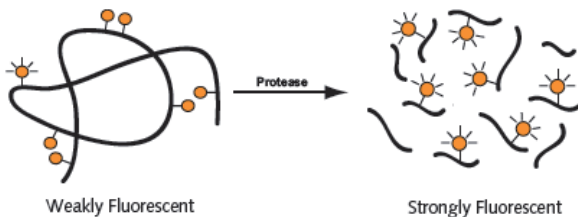


Figure 21: A schematic diagram of the quenched casein-fluorophore releasing fluorescence upon proteolytic cleavage.

Results

Sensolyte® Green Protease Assay

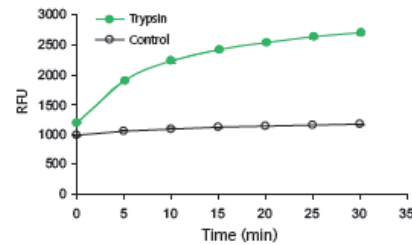


Figure 22: HiLyte Fluor™ 488-labeled casein was cleaved by 1 unit trypsin in assay buffer. The control wells contains HiLyte Fluor™ 488-labeled casein only, and no trypsin. Fluorescence was measured starting from Time 0, when trypsin was added, at Ex/Em=485±20 nm/528±20 nm (Flexstation 384II, Molecular Devices). Samples were done in duplicates.

Sensolyte® Red Protease Assay

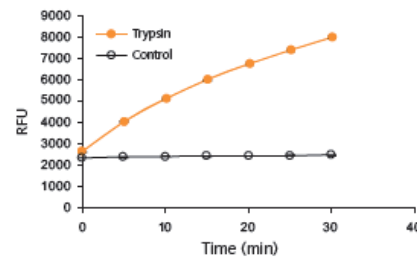


Figure 23: 5(6)-TAMRA labeled casein was cleaved by 1 unit trypsin in assay buffer. The control wells contain 5(6)- TAMRA-labeled casein only, and no trypsin. Fluorescence was measured starting from Time 0, when trypsin was added, at Ex/Em=530±25 nm/590±35 nm (Flexstation 384II, Molecular Devices). Samples were done in duplicates.

Assay kit overview

Enzyme/ Antibody	Detection	Product Detection	Reference
Generic Protease	Fluorimetric	Sensolyte® Green Protease Assay Kit	AS-71124
	Fluorimetric	Sensolyte® Red Protease Assay Kit	AS-71140

Product citations

Sensolyte® Red Protease Assay Kit
Groll, M. et al. Nature 452, 755 (2008).

Duolink®

New insights into proteins and protein interactions

For the first time, individual proteins, their interactions and modifications, can be accurately and objectively quantified in unmodified cells and tissues. Utilizing only a few cells, sub-cellular events, even transient or weak interactions, are revealed *in situ* and sub-populations of cells can be differentiated. Within hours, results from conventional co-immunoprecipitation and co-localization techniques can be confirmed.



Visualize & locate protein interactions

The Duolink kits are based on *in situ* PLA®, a technology that extends the capabilities of traditional immunoassays to include direct detection of protein interactions with unparalleled specificity and sensitivity. For the first time, any target can be readily detected and localized with single molecule resolution.

- ▣ Simultaneously visualize and locate protein-protein interactions

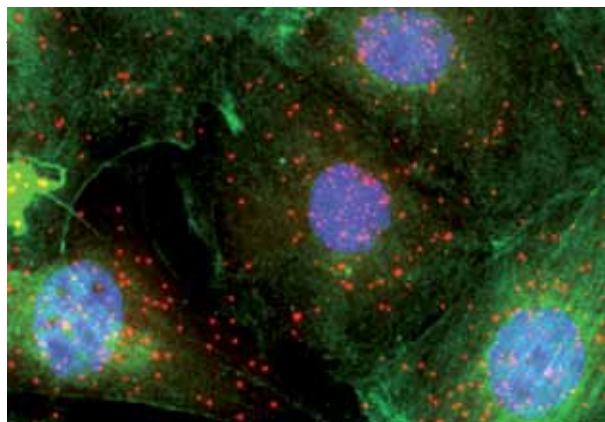


Figure 24. Single event resolution. Each red spot represents an SMAD 1/2/3 and SMAD 4 interaction in mouse embryonic fibroblasts. Green: FITC-anti-actin. Blue: DAPI stained nuclei.

- ▣ See sub-cellular events *in situ*

Retain protein localization. No modification of cells or tissue. No cell lysis, no over-expression, no interference from tags.

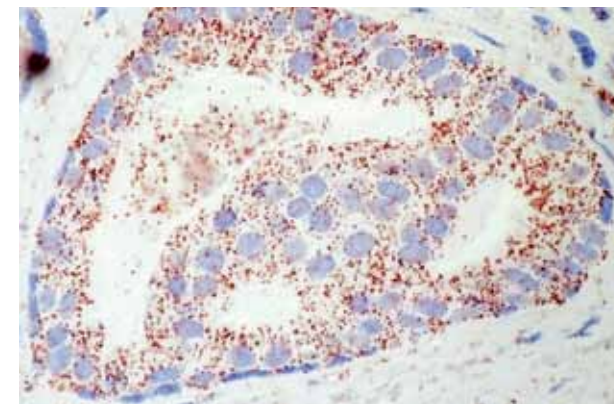


Figure 25. Breast cancer tissue (Duolink Detection Reagents Brightfield). Brown: HER2/HER3 interactions.

- ▣ Reveal transient and weak interactions *in situ*

- ▣ Study homodimeric protein complexes

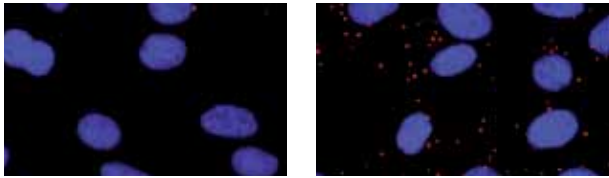
Duolink Probemaker enables the study of homodimeric complexes by allowing the use of same species monoclonal antibodies.

Simply include Probemaker probes, instead of PLA probes, in a Duolink kit.

- ▣ Work with endogenous proteins using only a few cells

- ▣ Differentiate between sub-populations of cells

Evaluate phosphorylation levels. Discrete fluorescent signals reveal phosphorylation of PDGF receptors.



Unstimulated control

BB(PDGF ligand) stimulated cells

Figure 26. Red: phosphorylated PDGF receptors.

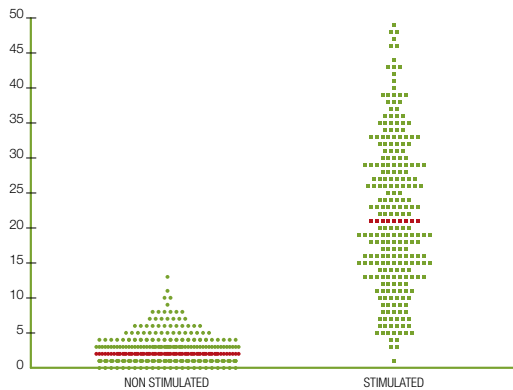


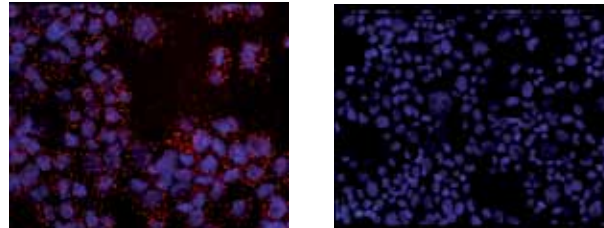
Figure 27. Dedicated Duolink ImageTool generates accurate data.

- ▣ See sub-populations of cells within tissue samples

- ▣ Confirm conventional co-ip and co-localization results - in one step

- ▣ Accurately quantify expression levels

Discrete fluorescent signals reveal expression of EGF receptor in A431 cells.



Primary antibody against EGFR

Negative control
no primary antibody added

Figure 28. Red: EGF receptors. Blue: DAPI stained nuclei.

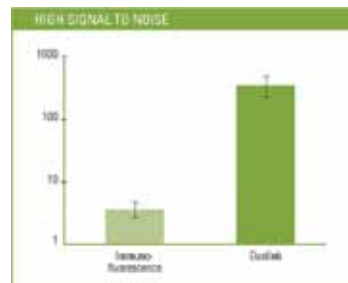


Figure 29. PLA signal-to-noise ratio 100 times higher than immunofluorescence (data derived from comparison with controls - no primary antibody added).

Additional applications

Using the in situ PLA technology you can also detect modifications such as phosphorylations or the expression of a single protein.



Detect and quantify protein phosphorylation

Detection of a modified protein such as a phosphorylated receptor can be done using one primary antibody against the receptor and the other against the phosphorylated site.

Detect and quantify protein expression

Detection of a single protein with high sensitivity can be done using only one primary antibody. Use PLA probes PLUS and MINUS against the same species.

Detect and quantify protein expression

Detection of a single protein with high sensitivity and specificity. Use two primary antibodies from different species against different epitopes of the same protein.

The Duolink® solution

Duolink II Starter kit

The Duolink® II Starter kit includes:

- ▣ PLA probe anti-Mouse MINUS (30 reactions)
- ▣ PLA probe anti-Rabbit PLUS (30 reactions)
- ▣ Detection Reagents Red or Orange (30 reactions)
- ▣ Wash Buffer for fluorescence
- ▣ Mounting medium with DAPI

Build your own kit

Each Duolink® *in situ* PLA protein detection kit consists of customizable kit components and can be configured to meet specific assay requirements. In addition to the kit components you will also need two primary antibodies raised in different species.



Building a kit that perfectly matches your need is easily done in four steps

- ▣ Choose PLA probe PLUS against the species one of your primary antibody has been raised in or Probemaker PLUS
- ▣ Choose PLA probe MINUS against the species the other primary antibody has been raised in or Probemaker MINUS

- ▣ Choose detection reagents depending on your mode of detection, fluorescence or chromogenic
- ▣ Choose the optimal accessories for your Duolink application

All necessary diluent solutions, enzymes and reagents needed to perform the Duolink assay are included in the kit components together with detailed step-by-step instructions.

Customizable kit components



- ▣ PLA probe PLUS; anti-Mouse PLUS, anti-Goat PLUS or anti-Rabbit PLUS
- ▣ PLA probe MINUS; anti-Mouse MINUS, anti-Goat MINUS or anti-Rabbit MINUS
- ▣ Probemaker PLUS and MINUS enables you to "make your own" PLA probes by conjugating the PLA oligonucleotide arms directly to antibodies
- ▣ Detection Reagents; Orange, Red, Far red or Green for fluorescence microscopy
- ▣ Detection Reagents Brightfield for brightfield microscopy

Recommended accessories



- ▣ Wash Buffers for fluorescence or brightfield – ready-to-use powder for all wash buffers
- ▣ Control Kit – reference slides, primary antibody and PLA probes to use as positive controls with fluorescence-based assays
- ▣ Mounting Medium with or without DAPI nuclear stain to preserve fluorescence signals
- ▣ Brightfield Mounting Medium for chromogenic-based assays
- ▣ Duolink Image Tool for image evaluation and result quantification

Results within one day

Preparation

Preparation and incubation of primary antibodies and PLA probes

STEP 1. Fix cells or tissues onto microscope slide, add blocking solution.



Typical starting materials are adherent cells, cytospin preparations or tissue sections on a glass slide, fixed, pretreated and blocked with a blocking reagent according to the requirements of the primary antibodies used.

STEP 2. Wash and add two primary antibodies



Primary antibodies must be of different species and each must recognize two different epitopes on the target molecules.

STEP 3. Wash and add the PLUS and MINUS PLA probes



PLA probes are secondary antibodies that bind to the primary antibodies. Each probe is equipped with a unique oligonucleotide.

Detection

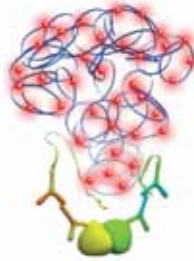
Amplification and detection of bound PLA probes

STEP 4. Wash and add Ligation solution



The Ligation solution, consisting of two oligonucleotides (illustrated as red bands) and Ligase, is added and the oligonucleotides will hybridize to the two PLA probes and join to a closed circle if they are in close proximity (<40 nm).

STEP 5. Wash and add Amplification solution



The Amplification solution, consisting of nucleotides and fluorescently labeled oligonucleotides (detection probes), is added together with Polymerase. A rolling circle amplification reaction generates a concatemeric DNA strand onto which the detection probes subsequently hybridize.

STEP 6. Wash, dry and mount slides



Use the appropriate Duolink® Mounting Medium to preserve and enhance the PLA signals. In order to stain the nuclei use Duolink Mounting Medium with DAPI. For brightfield applications use the nuclear stain included in the kit.

Analysis

Imaging and quantitative analysis

STEP 7. Review and capture images



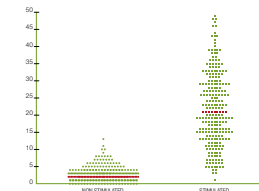
The PLA signal is recognized as a fluorescent or chromogenic spot. For fluorescent microscopy, use the appropriate filters for the detection fluorophore used. Export raw data into Duolink® ImageTool for objective quantification.

STEP 8. Obtain objective quantification using Duolink ImageTool



Automatic detection of nuclei and cytoplasmic regions. Compatible with data formats from major microscope vendors including Olympus, Leica, Nikon and Zeiss. Data can be exported into Excel for further evaluation.

STEP 9. Data analysis



Duolink® ImageTool enables single cell statistical analysis of expression levels in tissue or cell populations.

Duolink® PLA probes PLUS

Description	Size	Reference
Duolink® II PLA probe 30 anti-Mouse PLUS	30 reactions	AS-OK02-92001-0030
Duolink® II PLA probe 30 anti-Rabbit PLUS	30 reactions	AS-OK02-92002-0030
Duolink® II PLA probe 30 anti-Goat PLUS	30 reactions	AS-OK02-92003-0030
Duolink® II PLA probe 100 anti-Mouse PLUS	100 reactions	AS-OK01-92001-0100
Duolink® II PLA probe 100 anti-Rabbit PLUS	100 reactions	AS-OK01-92002-0100
Duolink® II PLA probe 100 anti-Goat PLUS	100 reactions	AS-OK01-92003-0100
Duolink® II PLA probe 500 anti-Mouse PLUS	500 reactions	AS-OK07-92001-0500
Duolink® II PLA probe 500 anti-Rabbit PLUS	500 reactions	AS-OK07-92002-0500
Duolink® II PLA probe 500 anti-Goat PLUS	500 reactions	AS-OK07-92003-0500
Duolink® II PLA probe 1000 anti-Mouse PLUS	1000 reactions	AS-OK08-92001-1000
Duolink® II PLA probe 1000 anti-Rabbit PLUS	1000 reactions	AS-OK08-92002-1000
Duolink® II PLA probe 1000 anti-Goat PLUS	1000 reactions	AS-OK08-92003-1000

Duolink® PLA probes MINUS

Description	Size	Reference
Duolink® II PLA probe 30 anti-Mouse MINUS	30 reactions	AS-OK02-92004-0030
Duolink® II PLA probe 30 anti-Rabbit MINUS	30 reactions	AS-OK02-92005-0030
Duolink® II PLA probe 30 anti-Goat MINUS	30 reactions	AS-OK02-92006-0030
Duolink® II PLA probe 100 anti-Mouse MINUS	100 reactions	AS-OK01-92004-0100
Duolink® II PLA probe 100 anti-Rabbit MINUS	100 reactions	AS-OK01-92005-0100
Duolink® II PLA probe 100 anti-Goat MINUS	100 reactions	AS-OK01-92006-0100
Duolink® II PLA probe 500 anti-Mouse MINUS	500 reactions	AS-OK07-92004-0500
Duolink® II PLA probe 500 anti-Rabbit MINUS	500 reactions	AS-OK07-92005-0500
Duolink® II PLA probe 500 anti-Goat MINUS	500 reactions	AS-OK07-92006-0500
Duolink® II PLA probe 1000 anti-Mouse MINUS	1000 reactions	AS-OK08-92004-1000
Duolink® II PLA probe 1000 anti-Rabbit MINUS	1000 reactions	AS-OK08-92005-1000
Duolink® II PLA probe 1000 anti-Goat MINUS	1000 reactions	AS-OK08-92006-1000

Duolink® Detection kits

Description	Size	Reference
Duolink® II 30 Detection Reagents Orange	30 reactions	AS-OK04-92007-0030
Duolink® II 30 Detection Reagents Red	30 reactions	AS-OK04-92008-0030
Duolink® II 30 Detection Reagents Brightfield	30 reactions	AS-OK04-92012-0030
Duolink® II 30 Detection Reagents Far Red	30 reactions	AS-OK04-92013-0030
Duolink® II 30 Detection Reagents Green	30 reactions	AS-OK04-92014-0030
Duolink® II 100 Detection Reagents Orange	100 reactions	AS-OK03-92007-0100
Duolink® II 100 Detection Reagents Red	100 reactions	AS-OK03-92008-0100
Duolink® II 100 Detection Reagents Brightfield	100 reactions	AS-OK03-92012-0100
Duolink® II 100 Detection Reagents Far Red	100 reactions	AS-OK03-92013-0100
Duolink® II 100 Detection Reagents Green	100 reactions	AS-OK03-92014-0100
Duolink® II 500 Detection Reagents Orange	500 reactions	AS-OK09-92007-0500
Duolink® II 500 Detection Reagents Red	500 reactions	AS-OK09-92008-0500
Duolink® II 500 Detection Reagents Brightfield	500 reactions	AS-OK09-92012-0500
Duolink® II 500 Detection Reagents Far Red	500 reactions	AS-OK09-92013-0500
Duolink® II 500 Detection Reagents Green	500 reactions	AS-OK09-92014-0500
Duolink® II 1000 Detection Reagents Orange	1000 reactions	AS-OK10-92007-1000
Duolink® II 1000 Detection Reagents Red	1000 reactions	AS-OK10-92008-1000
Duolink® II 1000 Detection Reagents Brightfield	1000 reactions	AS-OK10-92012-1000
Duolink® II 1000 Detection Reagents Far Red	1000 reactions	AS-OK10-92013-1000
Duolink® II 1000 Detection Reagents Green	1000 reactions	AS-OK10-92014-1000

Duolink® Probe Maker

Description	Size	Reference
Duolink® II Probe Maker PLUS - 20 µg	20 µg	AS-OK06-92009-0020
Duolink® II Probe Maker MINUS - 20 µg	20 µg	AS-OK06-92010-0020 270
Duolink® II Probe Maker PLUS - 5 x 20 µg	5 x 20 µg	AS-OK06-92009-20-5
Duolink® II Probe Maker MINUS - 5 x 20 µg	5 x 20 µg	AS-OK06-92010-20-5
Duolink® II Probe Maker PLUS - 10 x 20 µg	10 x 20 µg	AS-OK06-92009-2010
Duolink® II Probe Maker MINUS - 10 x 20 µg	10 x 20 µg	AS-OK06-92010-2010

Duolink® Accessories

Description	Size	Reference
Duolink® Mounting Medium 10ml	10ml	AS-OK05-80100
Duolink® Mounting Medium 5ml	5 ml	AS-OK05-80101
Duolink® Brightfield Mounting Medium - 40ml	40 ml	AS-OK05-80102-0040
Duolink® II Mounting Medium with DAPI	1 kit	AS-OK05-82040-0005
Duolink® II Wash Buffers for Fluorescence - 4 litres	4 litres	AS-OK05-82049-0004
Duolink® II Wash Buffers for Fluorescence - 20 litres	20 litres	AS-OK05-82049-0020
Duolink® II Wash Buffers for Brightfield - 4 litres	4 litres	AS-OK05-82047-0004
Duolink® II Wash Buffers for Brightfield - 20 litres	20 litres	AS-OK05-82047-0020
Duolink® II Control Kit	1 kit	AS-OK05-92011-0001
Duolink® ImageTool	1 software	AS-OK05-90806