

Cell adhesion study involves notably extracellular matrices, including collagen, Chondroitin (sulfated-GlycosAminoGlycans) and Elastin, and other metalloproteinases (MMPs). Following are remarkable assays for the study of cell adhesion and it's correlated events like migration and cancerogenesis.

Adhesion matrix extraction and detection (collagen, SGA, Elastin)

Sircol™ Soluble Collagen Assay

The Sircol™ Assay is a quantitative dye-binding method for the analysis of acid-soluble collagens extracted from mammalian tissues and collagens released into culture medium by mammalian cells during *in vitro* culture.

Salt-soluble, acid-soluble and pepsin-soluble forms of mammalian collagens, Types I to IV, can be measured.

- ◆ Working range of 0-50 µg collagen
- ◆ Assay time : 1 hour
- ◆ Assay sensitivity : 2.5 µg collagen.

Description	Cat.#	Qty
Sircol™ Soluble Collagen Assay Kits	U59610	1 kit (120 tests)
	U59611	1 kit (475 tests)
Acid-soluble Collagen Standard	BM1731	3 x 5 ml
Sterile ampoules of acid-soluble collagen (1.00 mg/ml)		

Components of the U59610 kit :

- Sircol Dye reagent (120 ml)
- Alkali Dye Release reagent (120 ml);
- Salt-soluble Collagen Precipitating reagent (12.5 ml);
- Acid-soluble Collagen Standard (5 ml of 1.00 mg/ml);

Blyscan™ Sulfated Glycosaminoglycan Assay

The Blyscan™ Assay is a quantitative dye-binding method for the analysis of sulfated proteoglycans (sPG) and glycosaminoglycans (sGAG), activator and potentiator of growth factors.

Have been measured following sGAG in soluble salt extracts from :

- ◆ *In vitro* studies where extracellular matrix components are released by live cells into the cell culture medium
- ◆ Elastic, fibrous and hyaline cartilages
- ◆ Arteries, lung, skin and other material containing extracellular matrix (connective tissue)
- ◆ Solid tumour specimens
- ◆ Cell and tissue extracts
- ◆ Aliquots from protein chromatography fractions

The assay also measures sGAG in amniotic fluid and urine samples, and may be adopted to detect and/or measure sGAG degradative enzymes, and to measure the N- and O-sulfated glycosaminoglycan ratio.

- ◆ Assay time : 1 hour
- ◆ Assay sensitivity : 0.5 µg sulfated glycosaminoglycan

Description	Cat.#	Qty
Blyscan™ Sulfated Glycosaminoglycan Assay kit	AA4880	1 kit (120 tests)
	AA4881	1 kit (475 tests)
Glycosaminoglycan Standard	BM1741	3 x 5 ml
Sterile ampoules of chondroitin-4 sulphate (100 µg/ml)		
sGAG Isolation & Concentration pack	BM1751	1 kit (100 runs)
Contains Buffered cetylpyridinium chloride and 2 M lithium chloride (Sufficient reagents for 100 samples)		

Components of the AA4881 kit :

- Blyscan™ Dye reagent (120 ml); Dissociation reagent (120 ml);
- Glycosaminoglycans Standard (5 ml of 100 µg/ml Ch-4-SO₄);
- Nitration reagents (15 ml);

Used to clean-up and concentrate test samples, prior to assay; and also for the pre-treatment of test samples with GAG level of less than 5 µg/ml

See also Ab Research Area #23 (Structural proteins)
See also Ab Research Area #8 (Cell adhesion)
See also Ab Research Area #2 (AngioGenesis/Histogenesis)
See also Cellspon support and devices for connective tissue research.
See also Cultrex™ Basement Membrane products
See also DIVAA™ Angiogenesis assay (#FX7300)
See also Fluorescent Protease Assays. (#BK9620, BK9630, HT1290)
See also Adiponectin EIA kit #BQ1620.

That's so easy to use :

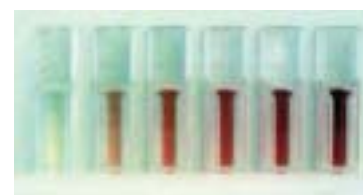
1. Mix sample and dye reagent.



2. Centrifuge sample and decant unbound dye Retain collagen bound dye.



3. Recover bound dye using Sircol dye release reagent Measure colour absorbance in cuvette or microwell plate.



Cell Biology - Assays Kits

Hormones - Peptides & Steroids

How to perform the Blyscan™ Assay :
reagent /Note : Pink blush in the Reference standard tube.

[1] Reagent blank (left) and Reference standard (right),
mixed with 1 ml Blyscan Dye reagent /



[2] Tubes centrifuged and supernatant removed.
Note : Purple pellet of dye-sGAG complex in Reference
standard tube.

[3] Tubes after addition of the Blyscan Dye Dissocia-
tion reagent.
Note : The pink/purple colour of the dye-sGAG complex
has been replaced by the blue colour of the "free" dye.



How the Fastin™ Assay work :

[1] Cold precipitated elastin (1 mg, overnight),
centrifuged and drain dried [2] Elastin-dye
complex after centrifuging and removing unbound
dye. Left : Reagent blank Right : Standard (50 µg)
[3] Recovery of elastin bound dye, after adding dis-
sociation reagent and centrifugation Left : Solvent/
buffer blank Middle : Reagent blank Right : Standard
(50 µg)

Fastin™ Elastin Assay

The Fastin™ Assay is a quantitative dye-binding method for the analysis of elastins released
into tissue culture medium and extracted from biological materials.

Elastin forms that can be measured :

- ◆ soluble tropoelastins
- ◆ lathyrogenic elastins
- ◆ insoluble elastins (as solubilised elastin polypeptides [α -elastin, κ -elastin])

Furthermore, elastase activity can be measured, using elastin as an enzyme substrate

- ◆ Assay time : 6 hours
- ◆ Assay sensitivity : 2.5 µg elastin

Description	Cat.#	Qty
Fastin™ Elastin Assay kit	Q99550	1 kit (120 tests)
	Q99551	1 kit (475 tests)

Components of the Q99550 kit :

Fastin Dye reagent (120 ml)
Precipitation reagent (120 ml)
Dye Dissociation reagent (120 ml)
 α -Elastin Standard (5ml of 100 µg/ml)

α -Elastin Standard	BM1761	3 x 5 ml
Sterile ampoules of α -elastin (100 µg/ml)		

Related products : stains (pathological investigation grade)

Amido Black 10B (dark blue)	07793A	25 g
Biebrich Scarlet Stain solution (violet)	BJ0001	200 ml
Mayer's Hematoxylin solution	82342A	500 ml
Ponceau (red, can be combined to nuclear staining by hematoxylin)	050261	500 ml

Matrix MetalloProtease Fluorimetric detection

MMP Fluorimetric Assays – EnzoLyte™ MMP Assay kits

- ◆ Accurate measurement of MMPs activity using proprietary FRET substrates
- ◆ Highly sensitive
- ◆ Easy to use
- ◆ Flexible format - 96 or 384 well.

The EnzoLyte™ fluorogenic assays are designed to assay MMP inducers and inhibitors. They detect individual MMP activity, in a variety of biological samples, being available for specificities MMP-1, 2, 3, 7, 8, 9, 12, 13, and 14, or detect simultaneously 16 specificities, or the global MMP activity.

EnzoLyte™ fluorogenic assays are performed in a convenient 96-well (100 assays) or 384-well (250 assays) microplate format, and suit high throughput screening of MMP. They use literature-documented MMP peptidic substrates with fluorescence resonance energy transfer (FRET) method. Two FRET systems are available :

- ◆ EDANS/DabcylPlus™ fluorescence resonance energy transfer (FRET) peptide, where DabcylPlus™ quenches the fluorescence of EDANS. Upon cleavage into two separate fragments by MMP, the fluorescence of EDANS is recovered, and can be monitored at excitation/emission wavelengths = 340 nm/490 nm. DabcylPlus™ is a quencher that is more water-soluble than Dabcyl, and it increases assay sensitivity by more than 10 fold as compared to an EDANS/Dabcyl FRET substrate, and so fold as compared to MCA/DNP substrate.
- ◆ 5-FAM/QXL™520 fluorescence resonance energy transfer (FRET) peptide, where the fluorescence of 5-FAM is quenched by QXL™520. Upon cleavage into two separate fragments by MMP, the fluorescence of 5-FAM is recovered, and can be monitored at excitation/emission wavelengths = 490 nm/520 nm (same filter set as FITC). With excellent fluorescence quantum yield and longer wavelength, 5-FAM shows less interference from autofluorescence of test compounds and cellular components. Compared to an EDANS/DABCYL FRET substrate, this 5-FAM/QXL™520 substrate provides better assay sensitivity.

Each kit contains :

Optimized FRET peptide substrate

Assay buffers

Fluorescence reference standard for calibration

See description below for generic, profiling and sampler kits

Fluorimetric Assay kits	EnzoLyte™ 490 EDANS/DabcylPlus™ FRET $\lambda_{exc}/\lambda_{em}$: 340 nm/490 nm	EnzoLyte™ 520 5-FAM/QXL™520 FRET $\lambda_{exc}/\lambda_{em}$: 490 nm/520 nm	Qty
EnzoLyte™ MMP-1 Assay Kit	BD6711	BN1951	100/250 tests
EnzoLyte™ MMP-2 Assay Kit	BD6721	BN1961	100/250 tests
EnzoLyte™ MMP-3 Assay Kit	BD6741	BN1981	100/250 tests
EnzoLyte™ MMP-7 Assay Kit	BD6751	BN2141	100/250 tests
EnzoLyte™ MMP-8 Assay Kit	BD6761	BN2171	100/250 tests
EnzoLyte™ MMP-9 Assay Kit	BD6771	BN2191	100/250 tests
EnzoLyte™ MMP-12 Assay Kit	BD6701	BN2211	100/250 tests
EnzoLyte™ MMP-13 Assay Kit	BD6781	BN2221	100/250 tests
EnzoLyte™ Generic MMP Assay Kit		BN2691	100/250 tests
EnzoLyte™ MMP Profiling Kit		BD6691	100/250 tests
EnzoLyte™ MMP Substrate Sampler Kit		BK9610	100/250 tests

Literature

- J. F. Woessner, Jr., *FASEB J.* 5, 2145-2154 (1991).
 Y. Okada, H. Nagase, E. D. Harris, Jr., *J. Biol. Chem.* 261, 14245-14255 (1986).
 A. Santala, J. Saarinen, P. Kovanen, P. Kuusela, *FEBS Lett.* 461, 153-156 (1999).
 F. Shabani, J. McNeil, L. Tippett, *Free Radic. Res.* 28, 115-123 (1998).
 V. Knauper, C. Lopez-Otin, B. Smith, G. Knight, G. Murphy, *J. Biol. Chem.* 271, 1544-1550 (1996).
 D. M. Bickett et al., *Ann. N. Y. Acad. Sci.* 732, 351-355 (1994).
 P. J. Kraft et al., *Connect. Tissue Res.* 42, 149-163 (2001).
 M. Aschi et al., *J. Comput. Aided Mol. Des* 16, 213-225 (2002).
 C. Bremer, C. H. Tung, R. Weissleder, *Acad. Radiol.* 9 Suppl 2, S314-S315 (2002).

Technical tip

Matrix metalloproteinases (MMP's)

Matrix metalloproteinases (MMP's) belong to a family of secreted or membrane-associated zinc endopeptidases capable of digesting extracellular matrix components. The importance of MMPs in tumor development and invasion as well as in other diseases is well known. These enzymes are responsible for the breakdown of connective tissues and are important in bone remodeling, menstrual cycle and the repair of tissue damage.

MMP-1 (collagenase) is involved in tumor development and metastasis and rheumatoid arthritis. It is proposed as a therapeutic target for these diseases.

MMP-2 (72-kDa gelatinase-A) is involved in tumor development and metastasis. It is proposed as a therapeutic target for cancer.

MMP-3 (stromelysin-1, transin-1) has been shown to be involved in tumor metastasis and rheumatoid arthritis. Therefore it is proposed as a therapeutic target for these diseases.

MMP-7 (matrilysin, PUMP) is proposed as a potential anti-cancer drug target.

MMP-8 (neutrophil collagenase) is proposed as a potential anti-cancer drug target.

MMP-9 (92-kDa gelatinase, collagenase-IV) is involved in a number of diseases such as cancer, angiogenesis, alopecia, and metastasis. It is proposed as a therapeutic target for these diseases.

MMP-12 (macrophage elastase) is involved in smoke-induced emphysema, tumor and other diseases. MMP-12 is secreted as a 54 kDa zymogen and becomes the mature 45 kDa active form after proteolytic cleavage.

MMP-13 (collagenase-3) is a member of the MMP family of extracellular proteases. Targets of MMP-13 include

collagen, gelatin, aggrecan, plasminogen and CXCL12. MMP-13 is secreted as a 60-kDa proenzyme (as measured by SDS-PAGE), and activated by cleavage to a mature 48-kDa MMP-13. MMP-13 is an important target for inhibitor screening due to its involvement in diseases such as cancer and arthritis

* 100 assays in 96 well microplate format, or 250 assays in 384 well format.

Cell Biology - Assays Kits

Hormones - Peptides & Steroids



Structure model of Metalloproteases (MMP-3 and TIMP-1).

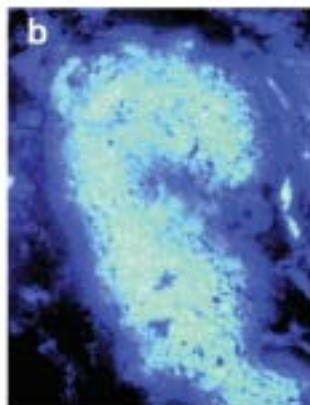
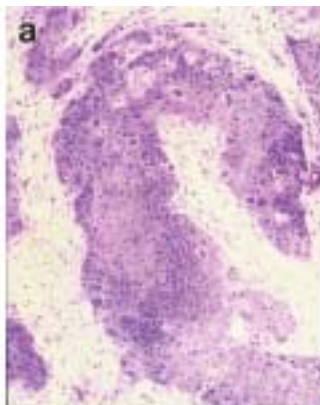


Fig. 1 Squamous cell carcinoma of oral cavity protease activity expression
a) HE stained
b) FIZ, Amido Black stained

EnzoLyte™ 520 Generic MMP Assay Kit

The EnzoLyte™ 520 Generic MMP Assay Kit is optimized to detect the activity of different MMPs with a 5-FAM/QXL™520 FRET peptide **polyspecific** for MMP-1, 2, 3, 7, 8, 9, 12, 13, and 14. Compared to the EnzoLyte™ 520 kits designed for individual MMP, this Generic MMP Assay kit is 5 to 20 fold more sensitive. This kit is ideal for detecting generic MMP activity in biological samples or for high throughput screening of MMPs' inducers and inhibitors using purified MMPs.

EnzoLyte™ 520 MMP Profiling Kit

The EnzoLyte™ 520 MMP Profiling Kit contains two 96-well plates pre-coated with sixteen different FRET peptide substrates. It provides a convenient platform for profiling substrate specificity for MMPs. 5-FAM and QXL™520, a pair of optimal fluorophore and quencher, are used in these FRET peptides.

EnzoLyte™ 520 MMP Substrate Sampler Kit

The EnzoLyte™ 520 MMP Substrate Sampler Kit utilizes a series of 16 FRET peptide substrates. It provides a convenient platform for profiling substrate specificity of MMPs and optimizing assay condition for MMPs. 5-FAM and QXL™520, a pair of optimal fluorophore and quencher, are used in these FRET peptides. Kit size : 16 x 20 assays

MMP Film in situ Zymography (FIZ)

A breakthrough for expression and tissue localization of MMP !

Benefits

- ◆ Very easy to use
- ◆ Visualize directly the localization of proteases in tissues or cells

The technique features strike those of immunological staining or in situ hybridization. It is also very simple. Significant quantitation can be obtained using digital analysing systems.

Applications

MMP in situ Zymo-Film was developed for observation of the enzyme activity of matrix metalloproteinases (MMPs) in tissue specimens, which are known as an indicator of carcinogenesis and other physiological conditions. To distinguish MMP activity from the other proteinase activity in the tissue, the experiment is performed simultaneously on MMP-PT slides in situ Zymo, which gelatin layer contains 1,10-Phenanthroline as a MMP inhibitor. several other applications are possible. Please see Nemori paper at <http://www.wako-chem.co.jp/siyaku/info/ibd/pdf/MMP-Nemori.pdf>.

Description	Cat.#	Qty
MMP in situ Zymo-film	BI9881	50 films
MMP-PT in situ Zymo-film	BI9891	50 films

Zymo-films are made under the license of Fuji Photo Film Co., Ltd. (Japan)

Membrane Fluidity Kit

Allows quantitative monitoring of membrane fluidity in cell membranes, micelles, and vesicles through use of a lipophilic pyrene probe.

The kit use the PDA probes that undergo eximer formation upon spatial interaction with lipids, with a dramatic shift to the red longer wavelengths, quantitative monitoring of the membrane fluidity is obtained by measuring the ratio of monomer (EM max. 372 nm) to eximer (EM 470 nm) fluorescence These measurements can provide kinetic information, as well as *in vivo* monitoring of cellular function by both flow cytometry and microscopic analysis.

Description	Cat.#	Qty
Membrane Fluidity Kit	BP8931	1 kit

Literature

- "Measurement of membrane fluidity of polymorphonuclear leukocytes by flow cytometry." Masuda M, Kuriki H, Komiyama Y, Nishikado H, Egawa H, Murata K. J. Immunol. Meth. 96(2) : 225-231 (1987).
 "Membrane fluid properties of cord blood mononuclear leucocytes : association with increased insulin receptors." Neufeld ND, Corbo LM. Pediatric Res. 18(8) : 773-778 (1984).
 "Effect of membrane fluidizers on the number and affinity of chemotactic factor receptors on human polymorphonuclear leukocytes." Tomonaga A, Hirota M, Snyderman R. Microbiol. Immunol. 27(11) : 961-972 (1983).

Contains : Fluorescent Lipid reagent (2 ml 10 µM of PDA)
 Reference standards
 Buffer solution media
 Pluronic® F-127

Technical tip

Overview

The dynamic properties of the cell membrane and cytoplasmic microtubules and microfilaments, as well as the dynamic movement of lipids in micelles and vesicles is of importance in such diverse areas as activation of polymorphonuclear leukocytes and chemotaxis, activation of membrane enzyme systems and the specific assembly or mobilization of microtubules and microfilaments, enhancement of the affinity of chemoattractant receptors, as well as being associated with a variety of pathological syndromes related to membrane fluidity.