funakoshi
FRONTIERS IN LIFE SCIENCE

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Bin*d*COL, biotin-conjugated <Denatured Collagen Detection Reagent>

Catalog NO. FDV-0035

Research use only, not for human or animal therapeutic or diagnostic use.

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Product Background

Collagen is the most abundant component of the extracellular matrix (ECM) and constitutes a large protein family in mammals. Collagen family shares unique triple-helical structures (Figure 1, left) consisting of three polypeptides that contain frequent repeats of Gly-Pro-Hyp (4-hydroxyproline) triplets. In human, 27 types are identified as the collagen family. Among them, types I, II and III occupy the majority (~90%) of the family in the body. Collagens have various functions from regulation of cellular activities, such as cell adhesion, migration and invasion, to mechanical support of tissues. In these processes, the dynamic remodeling of collagens including *de novo* production and degradation/denaturation is tightly regulated. Abnormal regulations of collagen-remodeling derived from excess production of unfolded or misfolded collagens or highly degradation of collagens leads to pathological conditions. Fibrosis in liver, osteoarthritis in cartilage, atherosclerosis in blood vessels and abnormal invasion of cancer cells are well-known examples of imbalance of collagen remodeling. Recent evidences indicate the increased amount of denatured collagen is associated with these diseases.

Denatured collagen (dCOL) is the loss of triple helical structure induced by heat, proteases, mechanical stress, and chemical modification (Figure 1, right). Once triple helical structures are destroyed, each polypeptide cannot re-organize the triple-helical structure. As denatured collagen is one of the pathological markers, detecting method for denatured collagens will be a good tool for pathophysiological research. Antibodies are one of the general tools for collagen research but cannot distinguish between native and denatured collagens. Only few antibodies are established to specifically detect denatured collagen, but they recognize only one specific collagen isoform. The detection probes satisfying following three points are highly desired. 1) Denatured collagen specific, 2) highly sensitive, and 3) broad cross-reactivity for various types of collagens. Recently, chemically synthesized collagen mimetic peptides (CMP) containing collagen-like amino acid sequences $(Gly\text{-}Pro\text{-}Hyp)_n$ are applied in detection of denatured collagens (Figure 2). CMPs selectively hybridize in the unfolded region of collagens. However, single-stranded **CMP**s (**ssCMP**) are preferentially self-assembled to homo trimer in water and it dramatically reduces binding affinity to denatured collagens. To avoid self-assembly of ssCMPs a pre-heating step is necessary. Pre-heating promotes dissociation of self-assembled trimers and increases the binding affinity of ssCMPs to denatured collagens. The experimental pre-heating process gives not only researcher's increased labor but also experimental bias in each trial,

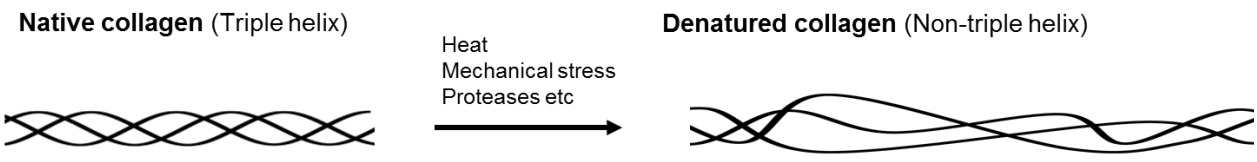


Figure 1. Overview of native and denatured collagens

ssCMPs are not sufficient tools for detecting various denatured collagens. To overcome this problem, Dr. Takaki Koide, Waseda University, developed the next generation denatured collagen detecting peptide. The novel peptide has highly unique structure which is a **s**trained **c**yclic **CMP** (**scCMP**) and has optimized anionic charges (anion-optimized scCMP). Due to strained cyclic structure and anionic charges, the self-assembly of the peptides are suppressed. The anion-optimized scCMP improves not only pre-heating step but also binding affinity for denatured collagen, over 10-fold higher than ssCMP. **Bin*d*COL**, a charge-optimized scCMP, shows dramatically higher sensitivity than ssCMP without pre-heating step. A biotin-conjugated **Bin*d*COL** can be flexibly used similar to primary antibody and easily detected by any fluorophore- or enzyme-linked avidin/streptavidin reagents.

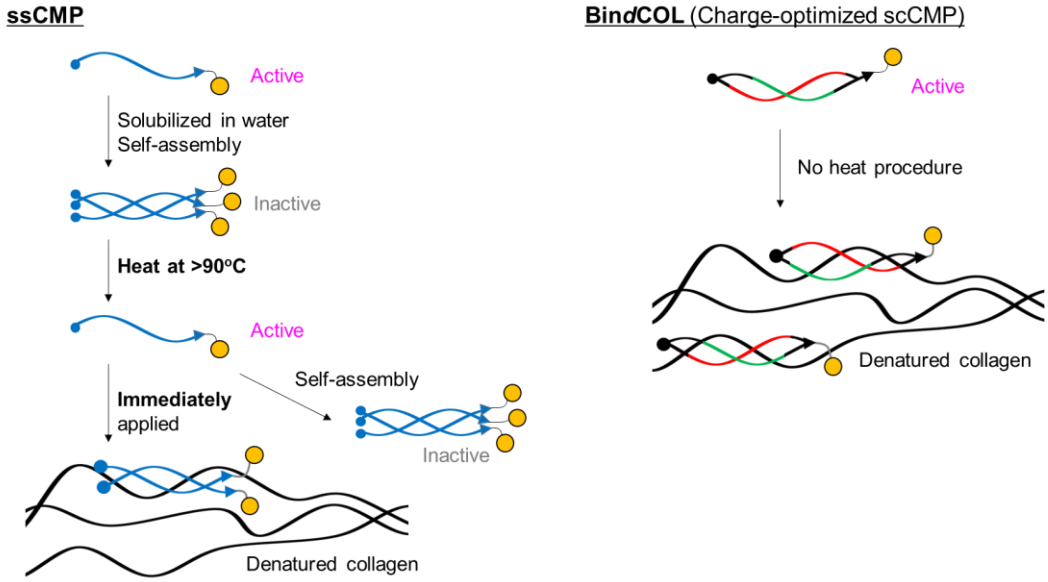


Figure 2. Comparison between ssCMP and Bin*d*COL

Collagen biosynthesis is another important process of dynamic collagen remodeling. Collagen polypeptides are firstly synthesized and post-translationally modified by prolyl hydroxylase in endoplasmic reticulum (ER). Three polypeptide chains cannot assemble triple helical structure by itself. Collagen-specific molecular chaperon, HSP47 promotes formation of trimers. Folded collagens are transferred to Golgi apparatus and secretory pathway. Abnormal collagen synthesis, maturation or secretory also induces some diseases. **Bin*d*COL** is an applicable detecting reagent for unfolded or misfolded collagens in ER under the biosynthetic pathway.

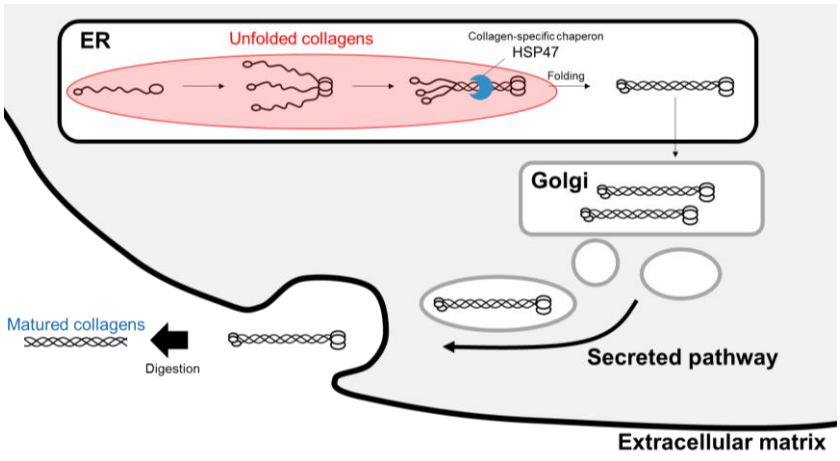


Figure 3. Biosynthesis of collagens and detection of unfolded and misfolded collagen in ER

Description

Catalog Number	: FDV-0035
Size	: 60 µg
Molecular weight	: ~4,700 Da
Probe structure	: Figure 4
Solubility	: Soluble in water
Label	: Biotin

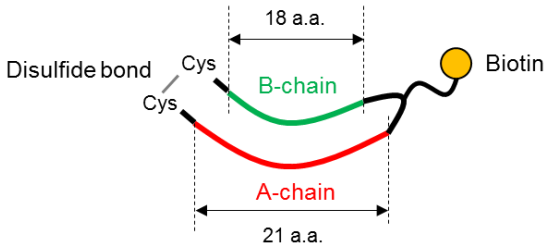


Figure 4. Image of Bin*d*COL-biotin structure

Applications

- Detection of denatured collagen
- Detection of intracellular unfolded and misfolded collagen
- Detection of total collagen in Western Blotting system

Storage

Store powder at **-20ºC**. Please avoid humid conditions.

Reconstitution and Note

Before use

The lyophilized peptide is a fluffy powder and may be attached to the tube wall or backside of tube lid. Before opening vial, please centrifuge vial and check the powder pellet bottom of vial.

Reconstitution

The reagent is well solubilized in water. Add 60 µL or 120 µL of water to the vial to prepare 1 mg/ml or 0.5 mg/ml stock solution, respectively, and vigorously shake tube to completely dissolve the powder. After reconstitution in water, make aliquots and store at -20 °C. Avoid repeated freeze-thaw cycles.

Note

This cyclic peptide has a disulfide bond (S-S bond) in the N-terminus (Figure 4). To protect S-S bond, all reducing reagents such as DTT, 2-ME and TCEP etc., are not compatible with this reagent. If S-S bond is cleaved by any reducing reagents, the binding affinity of the peptide will be dramatically decreased. Please carefully check if your assay buffers contain any reducing reagents.

How to use

* Following protocols are just general references. Each step depends on customer's experiments. Please optimize assay conditions for each experiment.

General procedure of denatured collagen detection by fluorescent imaging

1. Prepare 1-10 µg/ml Bin*d*COL reagent solution in PBS (or appropriate buffers)
2. Wash samples with PBS
3. Fix samples by 4% paraformaldehyde for 10-15 min
4. Wash samples with PBS several times
5. Block samples with any blocking buffers (3% BSA etc.) for 1 hour
6. Wash samples with PBS several times
7. Add 1-10 µg/ml Bin*d*COL reagent solution to samples for 1-2 hours.
8. Wash samples with PBS several times
9. Add fluorophore-conjugated streptavidin to the samples and incubate for 1 hour
10. Wash samples with PBS several times
11. Observe samples with fluorescence microscopies.

General procedure of denatured collagen detection by solid-phase binding (ELISA-like) assay

1. Coat biological samples containing denatured collagens on the multi-well plate such as 96 well plate.
2. Wash the wells with PBST several times
3. Block the wells with any blocking buffers (skim milk or BSA etc.) for >1 hour
4. Prepare 1-10 μg/ml Bin*d*COL reagent solution in PBS (or appropriate buffers)
5. Probe the wells with 1-10 μg/ml Bin*d*COL reagent solution for >1 hour
6. Wash the wells with PBST several times
7. Probe the wells with HRP- or AP-conjugated avidin/streptavidin for >1 hour
8. Wash the wells with PBST several times
9. Add HRP-substrate or AP-substrate to the wells
10. Measure colorimetric intensities by a microplate-leader

General procedure of detection of total collagen in Western Blotting system

1. Prepare cell or tissue lysates in SDS-sample buffer and boiled at 95°C for 5 min to denature collagen completely
2. Separate proteins by SDS-PAGE
3. Transfer proteins to nitrocellulose or PVDF membrane
4. Block the membrane with any blocking buffers (skim milk or 3% BSA etc.) for 1 hour.
5. Probe the membrane with 1-10 μg/ml Bin*d*COL reagent solution in blocking buffer for at least 1 hour.
6. Wash the membrane with PBST several times
7. Probe the membrane with HRP or AP-conjugated avidin/streptavidin for 1 hour.
8. Wash the membrane with PBST several times
9. Add HRP- or AP-substrate to the membrane
10. Detect the HRP or AP signal with chemical luminescence and colorimetric reagents.

NOTE: Under SDS-PAGE, all collagens are denatured by SDS. Please note "Denatured Collagen Detection Reagent" detects total collagen in the sample.

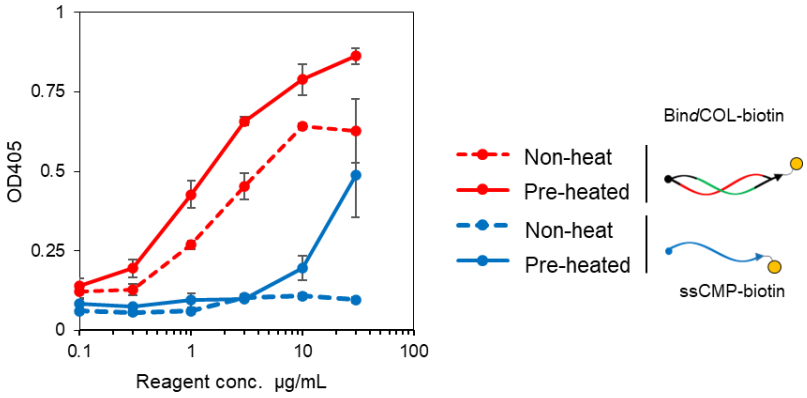
General procedure of intracellular unfolded and misfolded collagen staining

1. Wash cells with PBS several times
2. Fix samples by 4% paraformaldehyde for 10-15 min
3. Wash samples with PBS several times
4. Treat cells with 0.5% TritonX-100/PBS for 5 min *Cell membrane permeabilization is essential step.
5. Block samples with any blocking buffers (3% BSA etc.) for 1 hour
6. Wash samples with PBS several times
7. Add 1-10 μg/ml Bin*d*COL reagent solution in the blocking buffer to samples for 1-2 hours.
8. Wash samples with PBS several times
9. Add fluorophore-conjugated streptavidin in the blocking buffer to the samples and incubate for >1 hour
10. Wash samples with PBS several times
11. Observe samples with a fluorescence microscopy.

Reference data

Quantitative analysis of binding affinity of both ssCMP and **Bin*d*COL**

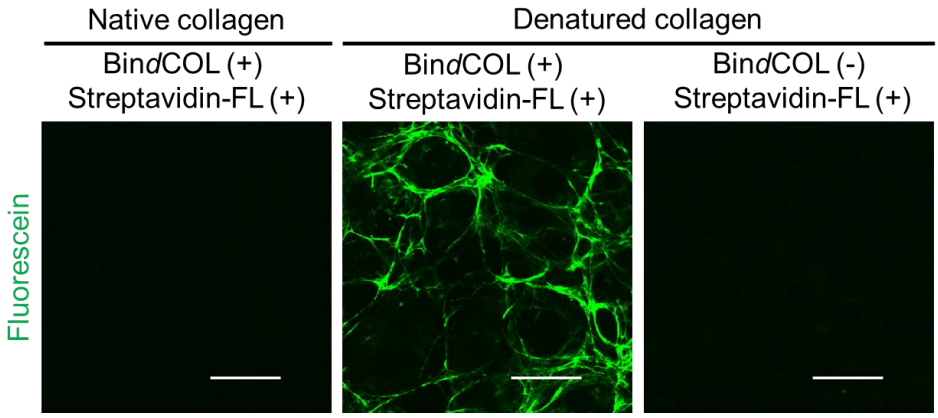
Wells on 96 well plate were pre-coated with heat-denatured collagen I. The wells were washed by ELISA buffer (20 mM HEPES-Na (pH 7.5), 100 mM NaCl, and 0.005% Tween-20) three times and blocked by 0.5% skim milk. After washing the wells, ssCMP-biotin or **Bin*d*COL**-biotin-containing solution were added into the wells and incubated for 1 hour. The wells were washed by ELISA buffer three times and probed with streptavidin-HRP conjugated. Binding intensity was estimated with a colorimetric substrate for HRP. **Bin*d*COL** under the non-heat condition shows 10-fold higher than ssCMP with pre-heating. This data indicated that **Bin*d*COL** shows great sensitivity compared with ssCMP.



Application data

Detection of denatured collagens derived from cultured cells

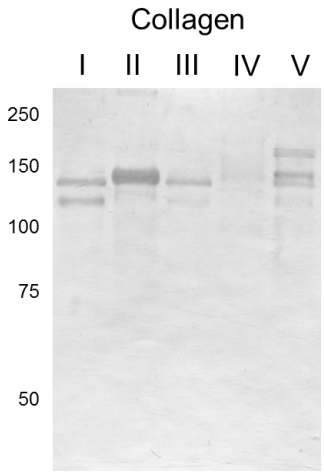
MEF cells were cultured at confluent condition for 3 days to produce collagens. For preparation of denatured collagens, the cell layers were treated with hot PBS (95°C) for 1 min. After the denaturation, they were fixed with 4% PFA and blocked by 3% BSA/PBS. They were incubated with 3 μg/ml of the **Bin*d*COL**-biotin in PBS for 1 hour, washed by PBS three times and incubated with streptavidin FITC-conjugated for 1 hour. They were washed and observed by confocal laser microscopy. The **Bin*d*COL**-biotin specifically detect denatured collagen, but no signal from native collagen was observed.



Detection of collagen members by Western Blotting system

Purified collagens (collagen I, II, III, IV and V) were separated by SDS-PAGE and transferred to a nitrocellulose membrane. The membrane was blocked by skim milk, probed with 5 μg/ml the reagent for 1 hour. After washing the membrane with TBST, the membrane was treated with streptavidin-AP conjugated to detect biotin-conjugated this probe. The **Bind*d*COL**-biotin could detect all collagens I-V by the SDS-PAGE/Western Blotting system.

NOTE: Under SDS-PAGE, all collagens were denatured. This result showed the reagent detect total collagen in the sample.

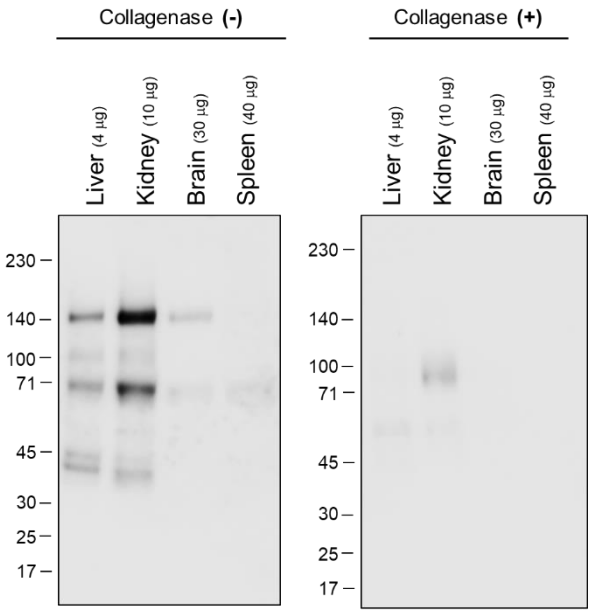


Detection of total collagen members in various tissues by Western Blotting system

Frozen mouse tissues (liver, kidney, brain and spleen) were homogenized by dounce-type homogenizer in homogenize buffer (50 mM HEPES (pH7.4), 100 mM NaCl, 0.32 M sucrose, 0.1% Triton-X100). Collagenase IV or water was added to each homogenate and the lysates were incubated for 2 hours at RT. After incubation, 10% SDS was added to each homogenate to be final 2% SDS concentration. The SDS-containing lysates were centrifuged and collected the supernatants to remove SDS-insoluble tissue debris. All samples were separated by SDS-PAGE and transferred to a PVDF membrane. The membrane was sequentially treated with blocking buffer (3% BSA in TBST) for 30 min, **Bind*d*COL**-biotin (0.4 μg/ml in TBST) for 1 hour and Streptavidin-HRP (0.05 μg/ml in TBST) for 1 hour. Finally, chemiluminescent signal was observed.

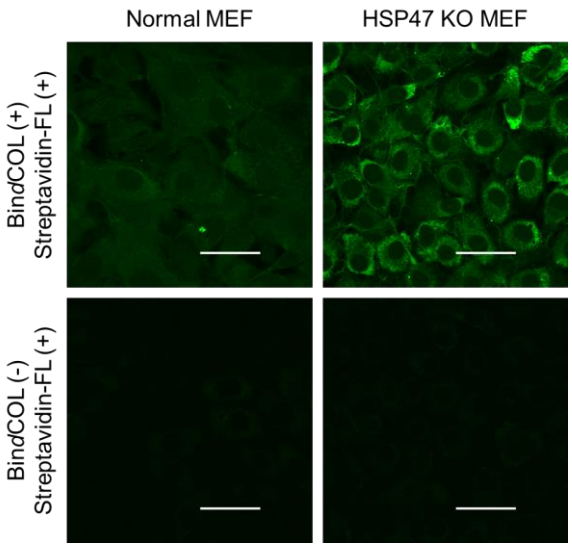
Bind*d*COL-biotin could detect collagen signals which can be degraded by collagenase treatment in all tissues tested in this experiment.

NOTE: Kidney in collagenase (+) still shows slight bands. Because molecular weight of these bands were different from original bands in collagenase (-), we concluded these bands were derived from partially cleaved collagens by collagenase.



Detection of intracellular unfolded and misfolded collagen

Normal MEFs and HSP47 KO MEFs were fixed with 4% PFA, permeabilized by 0.5% TritonX-100 and blocked by 3% BSA. After washing cells, the cells were probed with **Bind*d*COL**-biotin (3 μg/ml) for 1 hour, washed and further treated with streptavidin FITC-conjugated for 1 hour. After washing cells, the cells were observed in confocal microscopy. Collagen-specific chaperon HSP47-KO MEFs show higher signal from ER than normal MEFs. The **Bind*d*COL**-biotin is a valuable tool to estimate the amount of intracellular unfolded and misfolded collagen accumulation inside cells.



Reference

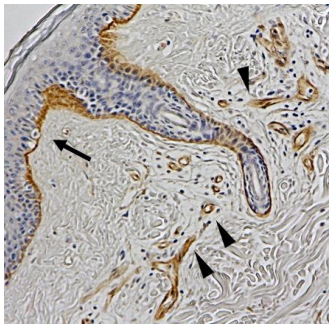
1. Takita, K. K., Fujii, K. K., Ishii, K., Koide, T., *Org. Biomol. Chem.*, **17**, 7380-7387 (2019) Structural optimization of cyclic peptides that efficiently detect denatured collagen.
2. Takita, K. K., Fujii, K. K. Kadonosono, T., Masuda, R., Koide, T., *ChemBioChem.*, **19**, 1613-1617 (2018) Cyclic Peptides for Efficient Detection of Collagen.

Related products

Catalog No.	Product name	Target	Application
FDV-0023	Anti-Laminin α3B, Human, Mouse-Mono (F7)	Laminin α3B	IHC, WB, IP, ELISA
FDV-0024	Anti-Laminin α3A, Human, Mouse-Mono (BG5)	Laminin α3A	IHC, WB, IP, ELISA
FDV-0025	Anti-Laminin γ2 N-terminal fragment, Human, Mouse-Mono (P2H)	Laminin γ2 N-terminal fragment	IHC, WB, ELISA
FDV-0026	Anti-Laminin 511, Human, Mouse-Mono (12D)	Trimeric Lm511 structure	IHC, WB, IP, ELISA

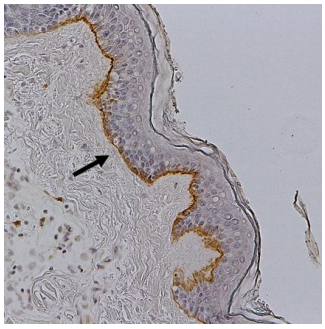
Anti-Laminin α3B (F7) #FDV-0023

Sample : normal human skin
Arrow head : vascular basement membrane
Arrow : epithelial basement membrane



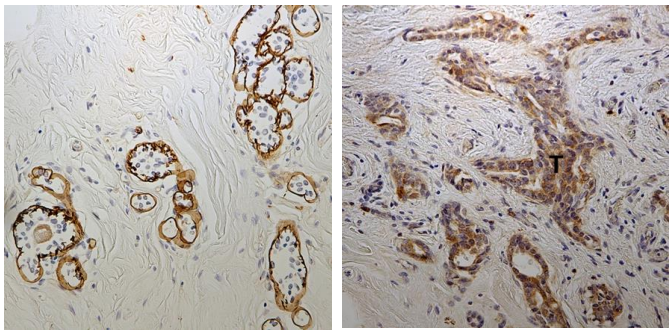
Anti-Laminin α3A (BG5) #FDV-0024

Sample : normal human skin
Arrow : epithelial basement membrane



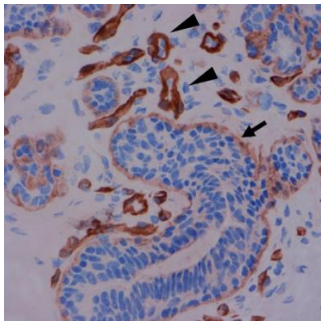
Anti- Laminin γ2 N-terminal fragment (P2H) #FDV-0025

Sample : human normal mammary gland (left), human breast cancer (right, T=tumor)



Anti-Laminin 511 (12D) #FDV-0026

Sample : human normal mammary gland
Arrow head : vascular basement membrane
Arrow : mammary gland basement membrane



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