

Laminin Marker Panel



Laminin subunit-specific antibodies

Laminin proteins are the major component of the basement membrane. They are crucial for a wide variety of biological processes, including cell adhesion, migration, differentiation and phenotype stability, embryonic development and angiogenesis. Laminins are heterotrimeric proteins that contain one alpha-, one beta- and one gamma-chain, found in five, four, and three genetic variants, respectively.

The goal of the Laminin Marker Panel has been to develop monoclonal antibodies that selectively recognize the specific subunits of the laminins. ELISA, Western Blot and IHC validation studies have been performed in order to select the clones with high specificity and no cross-reactivity to other subunits. Laminin antibodies can therefore be used as tools to identify the specific laminin subunits present in a tissue of interest. These antibodies may further contribute to understanding the roles and expression patterns of different laminins.

The Laminin Marker Panel consists of antibodies that allow for detection of 12 different laminin types, including Laminin-111, Laminin-211, Laminin-121, Laminin-221, Laminin-332, Laminin-311, Laminin-321, Laminin-411, Laminin-421, Laminin-511, Laminin-521 and Laminin-522.

The monoclonal antibodies within the Laminin Marker Panel have been developed in collaboration with BioLamina, using the same stringent conditions as for all PrecisA Monoclonals antibodies, which guarantees a secured continuity and stable supply.

Example: The Anti-LAMA5-, LAMB2- and LAMC1-specific antibodies

The Laminin antibodies have been carefully selected to recognize only one laminin subunit, allowing to use them as a tool to detect expression of specific laminins.

The examples here show the Anti-LAMA5 AMAb91124, Anti-LAMB2 AMAb91097 and Anti-LAMC1 AMAb91138 antibodies, targeting laminin alpha 5, beta 2 and gamma 1 chains respectively.

ELISA based functional characterization screen

All our laminin antibodies have been tested using ELISA to select and confirm that they are specific for only the corresponding laminin subunit and do not cross-react with other subunits. This has been performed using ELISA-plates coated with human recombinantly expressed laminins (not shown).

Subunit specificity confirmed by Western Blot

We have then further confirmed the subunit specificity in Western Blot using human recombinantly expressed laminins. Figure 1 shows that the Anti-LAMA5 AMAb91124, Anti-LAMB2 AMAb91097 and Anti-LAMC1 AMAb91138 only detect their specific subunits and do not display any cross-reactivity.

IHC to confirm protein expression in relevant tissues

Finally, the antibodies were tested by immunohistochemistry to confirm protein expression in relevant tissues and the absence of non-specific staining.

Positive IHC staining with Anti-LAMA5 AMAb91124, Anti-LAMB2 AMAb91097 and Anti-LAMC1 AMAb91138 on human tissues is shown in Figure 2.

Verification of subunit composition of laminins in tissue using multiplexed IHC

Laminin-521 is one of the first extracellular proteins expressed already by the pluripotent stem cells of the human embryo^{1,2}. Laminin-521 is crucially important for the development and function of several tissues, including heart muscle³, retinal epithelium⁴ and kidney⁵.

In adult kidney, laminin-521 is a key component of the glomerular basement membrane⁵. The cover image demonstrates how monoclonal antibodies against laminin alpha 5, beta 2 and gamma 1 subunits can be used to detect the presence of laminin-521 in kidney. The monoclonal antibodies used for this staining belong to different IgG isotypes, including IgG1, IgG2a and IgG2b. This enables multiplexed IHC with isotype-specific secondary antibodies to detect native laminin-521 on the same tissue section.



Cover image:

IHC-IF. (A) An overlay image visualizing the three laminin subunits, including LAMA5, LAMB2 and LAMC1 in human kidney. Arrows indicate the laminin-521 expression in the glomerular basement membrane. The individual images (B-D) show basement membranes staining using Anti-LAMA5 (AMAb91124) in blue (B), Anti-LAMB2 (AMAb91097) in green (C) and Anti-LAMC1 (AMAb91138) in red (D). Note that all three subunits are present in the basement membrane of glomerulus, while renal tubules express LAMA5 and LAMC1, but not LAMB2 subunit.

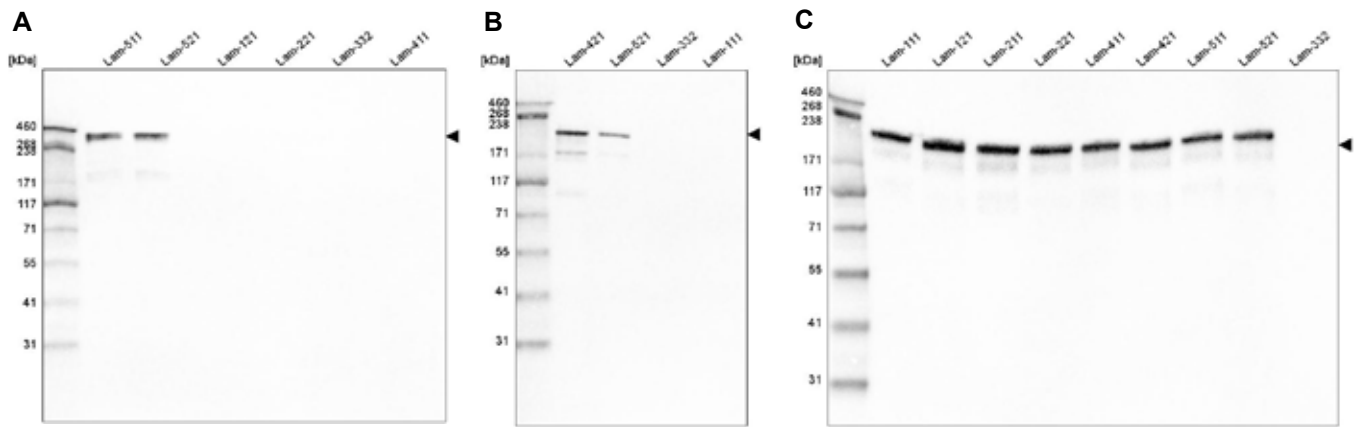


Figure 1

Western blot loaded with recombinantly expressed human laminins as indicated, showing the specificity of (A) Anti-LAMA5 (AMAb91124); (B), Anti-LAMB2 (AMAb91097); and (C) and Anti-LAMC1 (AMAb91138).

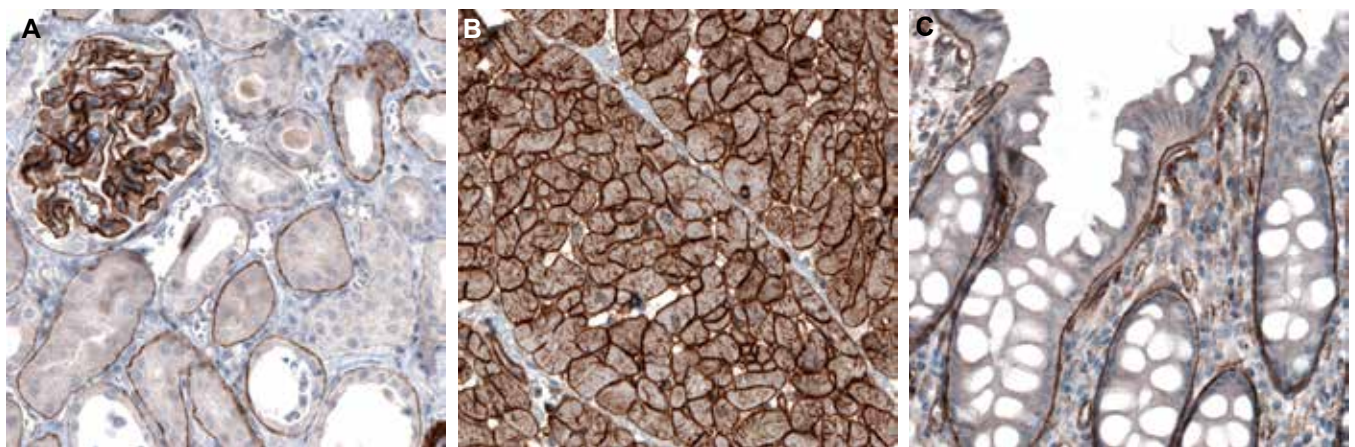


Figure 2

IHC-DAB. (A) The Anti-LAMA5 monoclonal antibody (AMAb91124) used for IHC staining of human kidney shows distinct staining of basement membranes in renal tubules and glomerulus. (B) IHC staining of human heart with Anti-LAMB2 monoclonal antibody (AMAb91097) shows strong membranous immunoreactivity in cardiomyocytes. (C) The Anti-LAMC1 monoclonal antibody (AMAb91138) shows positivity in basement membrane of glandular epithelium in human colon.

Table 1.

PrecisA Monoclonals Laminin Marker Panel

Product Name	Product Number	Epitope sequence	Validated Applications	Isotype
Anti-LAMA1	AMAb91091	DALEHLEDHQ	IHC, WB	IgG1
Anti-LAMA1	AMAb91117	LVPENFQDFHSKRQI	IHC, WB	IgG1
Anti-LAMA2	AMAb91166	DKLKPIKELEDNLKK	IHC, WB	IgG1
Anti-LAMA3	AMAb91123	EINSLQSDFT	IHC, WB	IgG1
Anti-LAMA4	AMAb91133	MANNLTNWSQNLQHF	IHC, WB	IgG2b
Anti-LAMA4	AMAb91134	GALARKSALKTRLSD	IHC, WB	IgG1
Anti-LAMA5	AMAb91124	RQGLVDRAQQ	IHC, WB	IgG1
Anti-LAMB1	AMAb91092	N.D.	IHC, WB	IgG1
Anti-LAMB2	AMAb91096	NANHALSGLERDRLA	IHC, WB	IgG1
Anti-LAMB2	AMAb91097	NANHALSGLERDRLA	IHC, WB	IgG2a
Anti-LAMB3	AMAb91160	ASEQALSAQEGFERI	IHC, WB	IgG1
Anti-LAMB3	AMAb91161	MTKQLGDFWTRMEEL	IHC, WB	IgG1
Anti-LAMC1	AMAb91136	N.D.	IHC, WB	IgG2b
Anti-LAMC1	AMAb91137	PACYRLVKDKVADHR	IHC, WB	IgG1
Anti-LAMC1	AMAb91138	AEVTDLNEVNNMLK	IHC, WB	IgG2b
Anti-LAMC1	AMAb91140	N.D.	IHC, WB	IgG1
Anti-LAMC2	AMAb91098	IQDTLNTLDGLLHLM	IHC, WB	IgG1

References

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