

Glomerular Basement Membrane (GBM; dissociated)

Antigen Specification

Product Number: 16800

Description:

Human $\alpha 3$ chain of collagen IV; identical with the antigen called "glomerular basement membrane antigen" (GBM). Recombinant antigen for solid (ELISA) and fluid phase diagnostic assays.

Immunological function:

Binds IgG-type human auto-antibodies.

Origin:

Recombinant. Expressed by recombinant baculovirus (*Autographa californica* multiple nuclear polyhedrosis virus; AcMNPV) infection of *Spodoptera frugiperda* Sf9 insect cells.

Expression construct:

cDNA coding for a minicollagen version of the human collagen IV $\alpha 3$ chain fused to a hexa-histidine purification tag. The term minicollagen designates the removal of most of the epitope-less triplehelical collagenous region (situated between the N-terminal 7S domain and the C-terminal noncollagenous NC1 domain), which is a requirement for recombinant production of this antigen.

Biochemical tests:

SDS-PAGE; Western blot with i: Goodpasture patient sera; ii: monoclonal anti-hexa-His-tag antibody.

Calculated molecular weight:

43,591 Dalton

Calculated isoelectric point:

pH 8.9

Immunological tests/Functionality:

Standard ELISA test (checkerboard analysis of positive/negative sera panels); immunodot test with positive/negative sera panels.

Recommended buffer/storage and handling conditions:

Recommendations for storage buffer: ionic strength between 50 and 100 mM, neutral to slightly alkaline pH and 4 M urea as dissociating agent. Storage temperature: -70° to -80° C. Repeated freeze/thaw cycles should be avoided.

Coating concentration:

0.12-0.5 μ g/ml (depending on the type of ELISA plate and coating buffer). Suitable for biotinylation and iodination.

CAUTION: It has been reported that the immunodominant epitope of GBM is a cryptic epitope that is not easily accessible to the corresponding autoantibodies. It is necessary to treat the protein under non-reducing conditions with a denaturant such as urea to unmask the epitopes (see Hellmark et al. in Autoantibodies, Peter, J.B. and Shoenfeld, Y., eds., Elsevier B.V., 1996, pp 291-298).

This GBM antigen product is produced in dissociated form and does not require additional unmasking of the epitope before coating.

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- Antigen Specification GBMdiss_16800_100414