

## 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 in vitro research and manufacturing use only.

**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 hexahistidine 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 (purity > 80%); Western blot with i: anti-GBM autoantibody-positive sample; ii: monoclonal anti-His-tag antibody.

**Calculated molecular weight:**

44 kDa

**Calculated isoelectric point:**

pH 8.9

**Immunological tests/Functionality:**

Standard ELISA test (checkerboard analysis of positive/negative samples); immunodot analyses with positive/negative samples.

**Recommended buffer/storage and handling conditions:**

Recommendations for storage buffer: neutral to slightly alkaline pH and 4 M urea as dissociating agent. Storage conditions: -70°C or below.

Repeated freeze/thaw cycles should be avoided.

**Coating concentration:**

0.12-0.5  $\mu\text{g}/\text{mL}$  (depending on the type of ELISA plate and coating buffer). Suitable for labeling of functional groups.

**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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