

## **Recombinant Human Glomerular Basal Membrane (GBM; dissociated)**

### **Antigen Specification**

**Product Number: 16800**

**Description:**

Human  $\alpha 3$  chain of collagen IV; identical with the antigen called "glomerular basal 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 (checker-board analysis of

positive/negative sera panels); Immuno-Dot test with positive/negative sera panels

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

Recommendations for storage buffer: ion strength ~50-100 mM, neutral to slightly alkaline pH and 4 M urea as dissociating agent. Storage temperature:  $-70^{\circ}$  to  $-80^{\circ}$  C. Repeated freeze/thaw cycles should be avoided.

**Coating concentration:**

0.12-0.5  $\mu$ 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. For manufacturers wishing to dissociate GBM immediately prior or during the coating process, DIARECT can provide an alternative undissociated GBM product (product number 15900).

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