Mouse Nephritis Reagents

Membranous nephropathy (MN) is a disease caused by granular subepithelial deposition of immunocomplexes along the glomerular basement membrane (GBM) in kidneys. Immunization with chemically modified cationic bovine serum albumin (cBSA, Catalog # 9058) is capable of inducing immune complex glomerulonephritis (ICGN) in BALB/c and ICR mice. Additionally, recent research has confirmed that cBSA from Chondrex, Inc. can induce ICGN in C57BL/6 mice due to its high isoelectric point (1). This finding enables the adaptation of this model to transgenic mice, leading to a better understanding of gene association in ICGN and the discovery of new therapeutic approaches. A Mouse Albumin Detection Kit (Catalog # 3012) is also available to evaluate ICGN severity (2,3). For more information about these products, please contact Chondrex, Inc. at support@chondrex.com.

Standard Protocol

BALB/c or ICR Mice

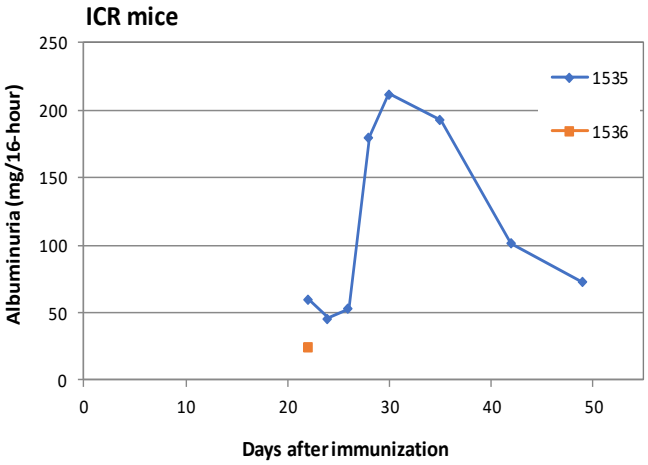
1. Immunize 100 µg cBSA (Cat # 9058) emulsified with 1 mg/ml Complete Freund's Adjuvant (Cat # 7008) at the base of the tail (1 x subcutaneous injection)
2. Wait 2 weeks
3. Inject 400 µg cBSA by intravenous injection every other day (5 x intravenous injections)
4. Obtain a 16-hour urine sample to evaluate the severity of nephritis
5. Measure albuminuria

Benefits:

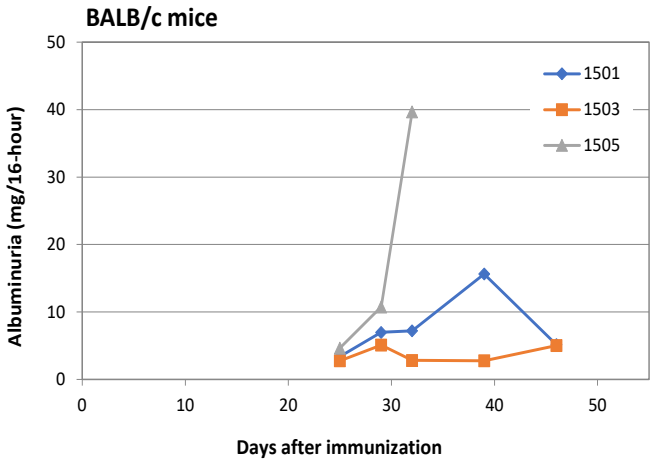
- Develop albuminuria in < 4 weeks
- Usage in common mouse strain (BALB/c)

Note:

Albuminuria in mice is defined when 1 mg or more of albumin is excreted in a 16-hour urine sample. Urine volumes vary among individual mice, therefore total amounts of protein excreted into a 16-hour urine sample should be determined instead of protein concentration for an accurate evaluation of nephritis severity.



Mouse # 1536 died on day 24 after the first urine collection. Mouse # 1535 developed severe albuminuria, peaking on day 32.



Mouse # 1505 died on day 30 after developing severe albuminuria. Albuminuria lasted for 3 weeks after the last cBSA IV injection in the other two mice.

Mouse Nephritis Reagents

Mouse Albumin Assay Kit (Catalog # 3012)

Proteinuria is a common symptom of nephritis in humans and experimental animals. Determining the total amount of protein (serum proteins such as globulins and albumin) excreted in a 16-hour urine collection period is distinctly more accurate than determining the individual urinary protein concentrations due to large variations in urine volume between individual animals. Moreover, mouse kidneys leak serum components such as bilirubin, resulting in overestimated urinary protein levels. On the other hand, albumin, a serum protein with a relatively small molecular weight, and typically the first protein observed in the urine when kidney dysfunction begins to develop, is a more suitable marker to evaluate the severity of nephritis in mouse models.

Bromophenol Blue (BPB) Protein Assay Kit (Catalog # 6026)

Urinary protein levels, a marker of renal disease in rodents, are commonly determined by a simple dipstick method which can be affected by urine volume and color, leading to inaccurate results. Chondrex, Inc. provides a bromophenol blue (BPB) assay kit which is a simple, precise, and accurate alternative to determine proteinuria. Because BPB has a higher affinity to albumin than many types of globulins, this assay may better reflect glomerular albuminuria.

Comparing Both Assay Methods

Product	Specificity	Sensitivity	Sample Volume	Assay Time
Mouse Albumin Assay	Albumin Only	1.6 - 100 ng/ml	Less than 10 µl	4 hours
BPB Protein Assay	Primarily Albumin	0.03 - 2 mg/ml	100 µl	10 minutes

References

1. P. Sun, S. Feng, Q. Guan, H. Adomat, S. Barbour, M. E. Gleave, C. Y. C. Nguan, W. Xu, C. Du, Clusterin Deficiency Predisposes C57BL/6j Mice to Cationic Bovine Serum Albumin-Induced Glomerular Inflammation. *J. Inflamm. Res.* **13**, 969–983 (2020).
2. Y. S. Huang, H. Y. Hsieh, H. M. Shih, H.-K. Sytwu, C.-C. Wu, Urinary Xist is a potential biomarker for membranous nephropathy. *Biochemical and Biophysical Research Communications*. **452**, 415–421 (2014).
3. Y. S. Huang *et al.*, Role of melatonin receptor 1A and pituitary homeobox-1 coexpression in protecting tubular epithelial cells in membranous nephropathy. *Journal of Pineal Research*. **65**, e12482 (2018).