

## Monoclonal Antibodies for Studying Alport Syndrome

Catalog # 70751 and 70781

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### INTRODUCTION

In the kidney, the glomerular basement membranes (GBM) contain type-IV collagen which consists of trimers of  $\alpha 3$ ,  $\alpha 4$ , and  $\alpha 5$  chains ( $\alpha 3(\text{IV})$ ,  $\alpha 4(\text{IV})$ , and  $\alpha 5(\text{IV})$ , respectively) and heterotrimers of  $\alpha 1(\text{IV})$  and  $\alpha 2(\text{IV})$  chains (1). Genetic mutation of the  $\alpha 5(\text{IV})$  chain, which is regulated by the X chromosome, disrupts the formation of the  $\alpha 3$ -4-5 networks and leads to abnormal GBM development. This structural defect causes nephritis known as X-linked Alport syndrome. Alport syndrome can be diagnosed by pathogenesis tests which detect the type IV collagen mutation by immunostaining the aforementioned collagen chains, and can be further evaluated by kidney function tests which determine urinary protein levels (2).

Additionally, skin epidermal basement membranes (EBM) similarly contain type-IV collagen composed of heterotrimers of  $\alpha 1(\text{IV})$  and  $\alpha 2(\text{IV})$ , and heterotrimers of  $\alpha 5(\text{IV})$  and  $\alpha 6(\text{IV})$ . Therefore, determining  $\alpha 5(\text{IV})$  chain mutations in skin biopsy sections by immunostaining is a useful method for diagnosing Alport syndrome due to the minimally invasive method of sample preparation (3).

Chondrex, Inc. provides the two epitope-defined biotinylated rat monoclonal antibodies (mAbs) against the  $\alpha 2(\text{IV})$  chain (catalog # 70751, H25) and the  $\alpha 5(\text{IV})$  chain (catalog # 70781, H53). Immunostaining using the anti- $\alpha 5(\text{IV})$  mAb in Alport syndrome patient samples shows abnormal distributions and discontinuous patterns of type IV collagen due to the mutation of the collagen chains in the GBM while immunostaining with the anti- $\alpha 2(\text{IV})$  mAb shows normal structural patterns (4, 5). The following immunostaining protocol using these mAbs may be helpful for studying the mechanism and pathogenesis of Alport syndrome (1, 6).

### PROCEDURE

#### A. CRYOSTAT SECTIONS

An immunostaining method for  $\alpha 2(\text{IV})$  and  $\alpha 5(\text{IV})$  on cryostat sections by biotinylated rat monoclonal antibodies (H25: Catalog # 70751 and H53: Catalog # 70781). The following protocol may need to be optimized depending on sample type.

1. Cut two 3  $\mu\text{m}$  thick cryostat sections, air dry, and fix in acetone for 10 minutes at room temperature.
2. Wash two slides in 0.05M phosphate saline buffer, pH 7.4 for 5 minutes.
3. Block the two slides with 1% casein in 0.05M phosphate saline buffer, pH 7.4 for 30 minutes.
4. Incubate the two slides with the following biotinylated monoclonal antibodies for 1 hour:
  - a. H25 (diluted 1:25 in 1% casein in 0.05M phosphate saline buffer, pH 7.4 )
  - b. H53 (diluted 1:25 in 1% casein in 0.05M phosphate saline buffer, pH 7.4).
5. Wash the two slides in 0.05M phosphate saline buffer, pH 7.4 for 10 minutes.
6. Incubate all four tissue slides with FITC-labeled Avidin in 1% casein in 0.05M phosphate saline buffer, pH 7.4 for 1 hour.
7. Wash in 0.05M phosphate saline buffer, pH 7.4 for 10 minutes and add mounting media containing p-Phenylenediamine to delay fluorescence quenching.

## **B. PARAFFIN-EMBEDDED TISSUE SECTIONS**

An immunostaining method for  $\alpha 2$  (IV) NC1 and  $\alpha 5$ (IV) on formalin-fixed, paraffin-embedded tissue sections by biotinylated rat monoclonal antibodies (H25: Catalog # 70751 and H53: Catalog # 70781). The following protocol may need to be optimized depending on sample type.

1. Cut two 3-4  $\mu$ m thick formalin-fixed, paraffin-embedded tissue sections, deparaffinize, and rehydrate.
2. Immerse the sections with 0.2M HCl, pH 0.9 and heat in a small autoclave at 110-127°C for 6 minutes.  
(Alternatively, boil sections in citrate buffer, pH 2.0 in a microwave oven at 750W for 8 minutes followed by 350W for 15 minutes )
3. Rinse two slides in distilled water for 2 minutes.
4. Block the two slides with 1% casein in 0.05M phosphate saline buffer, pH 7.4 for 30 minutes.
5. Incubate the two slides with the following biotinylated monoclonal antibodies for 1 hour:
  - a. H25 (diluted 1:50 in 1% casein in 0.05M phosphate saline buffer, pH 7.4 )
  - b. H53 (diluted 1:50 in 1% casein in 0.05M phosphate saline buffer, pH 7.4).
6. Wash the two slides in 0.05M phosphate saline buffer, pH 7.4 for 10 minutes.
7. Incubate all four tissue slides with FITC-labelled Avidin in 1% casein in 0.05M phosphate saline buffer, pH 7.4 for 1 hour.
8. Wash in 0.05M phosphate saline buffer, pH 7.4 for 10 minutes and add mounting media containing p-Phenylenediamine to delay fluorescence quenching.

## **INTERPRETING STAIN RESULTS (4. 6. 7)**

In the kidney biopsy samples from normal patients, H25 and H53 linearly stain the glomerular basement membrane (GBM), the Bowman's capsules (BC), and the distal tubular basement membranes (dTBM) (Figure 1 Top). In males with X-linked Alport syndrome (XLAS) , H25 stains the tissue same as in normal patients, however, H53 fails to stain the GBM, BC, and dTBM (Figure 1 Middle). However, in females with XLAS, H53 discontinuously stains the GBM, but not the BC and dTBM. In autosomal recessive Alport syndrome (ARAS), although H25 stains the GBM, BC, and dTBM, H52 stains with a mosaic pattern in the BC and dTBM (Figure 1 bottom). Table 1 and Figure 1 show typical staining patterns in the three groups. Therefore, these two monoclonal antibodies work to distinguish XLAS and ARAS by immunohistochemical analysis of renal biopsies.

Using skin biopsy samples, H25 stains the epidermal basement membrane (EBM) in all patients, however, H53 stains the EBM only in normal patients, and failed to stain the EBM in XLAS and ARAS patients (Table 1).

Note: the staining results may vary due to sample preparation. Chondrex, Inc. recommends establishing your own control references.

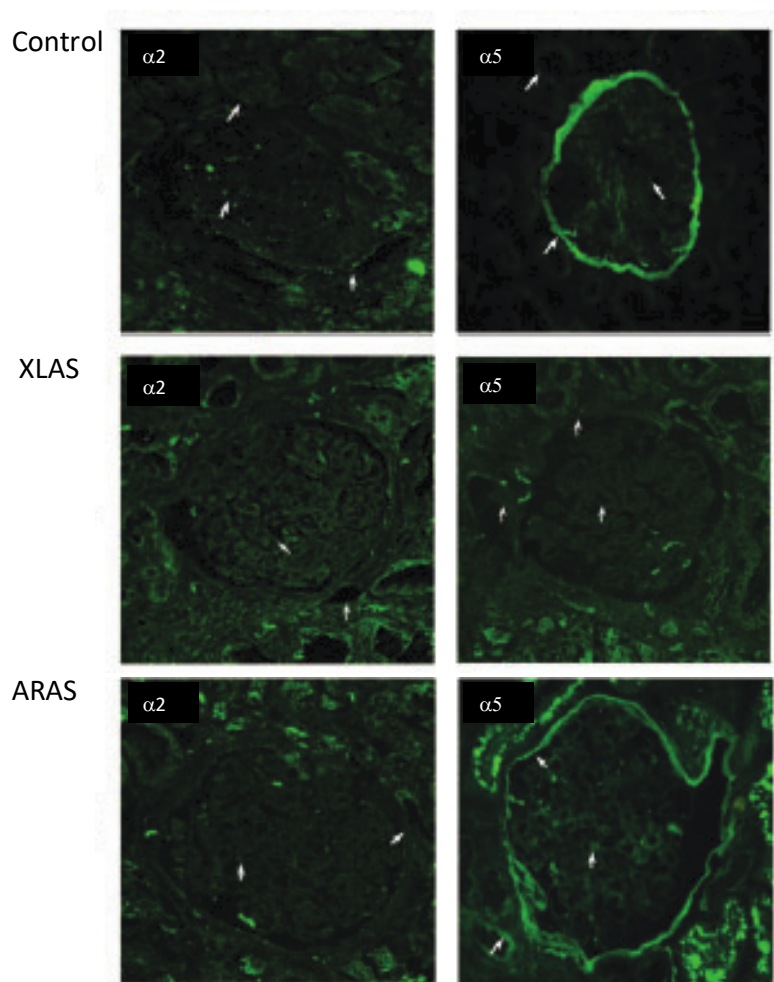
Table1. Typical staining results using anti-type IV collagen antibodies in patients

	Renal Tissue				Skin Tissue	
	Alpha 2 chain		Alpha 5 chain		Alpha 2 chain	Alpha 5 chain
	GBM	dTBM	GBM	dTBM	EBM	EBM
Control	+	+	+	+	+	+
XLAS	+	+	-	-	+	-
ALAS	+	+	-	+	+	-

Note 1: +: Positive (+\*: Mosaic staining pattern), - : Negative

Note 2: GBM: Glomerular basement membrane, dTBM: distal tubular basement membrane  
EBM: Epidermal basement membrane

Figure 1. Typical staining patterns in patients



## REFERENCES

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