



**KAMIYA BIOMEDICAL COMPANY**

# Collagen Type IV Staining Kit

**For the immunohistochemical staining of collagen type IV  
alpha 1, 3 and 5 chains.**

**Cat. No. KT-153**

**For Research Use Only. Not for Use in Diagnostic Procedures.**

## PRODUCT INFORMATION

### Collagen Type IV Staining Kit

Cat. No. KT-153

#### PRODUCT

The K-ASSAY® Collagen Type IV Staining Kit is for the immunohistochemical staining of collagen type IV alpha 1, 3 and 5 chains. For research use only. Not for use in diagnostic procedures.

#### DESCRIPTION

Mutations in the gene for the alpha 5 chain of type IV collagen result in a lack of the alpha 5 (IV) chain from glomerular basement membrane (GBM) and the skin basement membrane as well as a lack of the alpha 3 (IV) chain from GBM. The staining and analysis should be performed by trained laboratory professionals.

#### COMPONENTS

- 200 µL Monoclonal mouse antibody, Anti-Collagen Type IV alpha 1 chain ( $\alpha$  1 chain mAb)
- 200 µL Monoclonal mouse antibody, Anti-Collagen Type IV alpha 3 chain ( $\alpha$  3 chain mAb)
- 100 µL Monoclonal rat antibody, Anti-Collagen Type IV alpha 5 chain ( $\alpha$  5 chain mAb)
- 1 x 23 mL Glycine/Urea Solution (Store in a freezer since urea slowly disintegrates.)

#### PROTOCOLS

##### METHOD 1

##### Staining of frozen sections by monoclonal antibodies:

1. 3 µm thick cryostat sections are cut, air-dried, fixed in acetone for 10 minutes.
2. Wash the three slides in PBS for 5 minutes and leave the MAB1 and MAB3 slides in PBS
3. MAB5 slide only: The section to be stained with MAB5 is incubated in the glycine/urea solution for 5 min at 4°C.
4. MAB5 slide only: Wash in sterile PBS for 10 min.
5. Incubate the three tissue slides for 1 hour with their respective monoclonal antibody:
  - a. MAB1 (diluted 1:25)
  - b. MAB3 (diluted 1:25)
  - c. MAB5 (diluted 1:50)
6. Wash in sterile PBS for 10 minutes.
7. Incubate the three tissue slides for 1 hour with their respective FITC-labeled secondary antibodies:
  - a. MAB1 and MAB3 tissue slides with Anti-Mouse FITC.
  - b. NOTE! MAB5 tissue slide with Anti-Rat FITC.
8. Wash in sterile PBS for 10 minutes and add mounting media containing p-phenylene diamine to delay fluorescence quenching.

##### METHOD 2

##### Staining method for type IV collagen $\alpha$ 1 chain mAb, $\alpha$ 3 chain mAb and $\alpha$ 5 chain mAb using immunoperoxidase on formalin-fixed, paraffin-embedded tissue using K-ASSAY® Collagen Type IV Staining Kit

1. For each tissue specimen to be analyzed, cut five 3-4 µm thick formalin-fixed, paraffin-embedded tissue sections, deparaffinise and rehydrate.
2. **Antigen demasking** –  
Microwave boiling in citrate buffer, pH 2 at 750W for 8 minutes followed by 350W for 15 min. Use the citrate buffer pH6 (Dako S2031) adjusted with 1M HCl.

3. Rinse the five slides in distilled water for 2 minutes. MAB1, MAB3, and negative control slides proceed to step 6.
4. MAB5 slide only: The section to be stained with MAB5 is incubated in the glycine/urea solution for 10 minutes at 4°C.
5. MAB5 slide only: Rinse in distilled tap water for 2 x 2 min.
6. Endogenous peroxidases are blocked by incubating three slides with 1.5% H<sub>2</sub>O<sub>2</sub> in TBS for 10 min.
7. Rinse the five slides in tap water for 5 min, followed by distilled water for 1 minute.
8. Incubate the three tissue slides for 1 hour with their respective monoclonal antibody:
  - a. MAB1 (diluted 1:50)
  - b. MAB3 (diluted 1:50)
  - c. MAB5 (Diluted 1:100)
  - d. Negative control, mouse (PBS)
  - e. Negative control, rat (PBS)
9. Rinse the three slides in distilled water for 1 minute.
10. Finalize the staining of the three slides according to the instructions of your respective detection systems:
  - a. MAB1 and MAB3 tissue slides with the anti-mouse detection system.
  - b. Note! MAB5 tissue slide with the anti-rat detection system.

## INTERPRETATION OF STAINING

For proper interpretation a negative (without primary antibody) and a positive control ( $\alpha$  1 chain mAb) should be run alongside samples. The positive control serves to indicate that sample processing and staining were carried out correctly and should show intense staining along basement membranes, in the glomerulus mainly to the mesangial matrix and the subendothelial aspects of the GBM. The negative control serves to assess non-specific staining which should be taken into consideration when interpreting results.

$\alpha$  3 chain mAb and  $\alpha$  5 chain mAb normally stain the entire thickness of the GBM as well as the distal tubular BM. In samples with X-linked disease, the  $\alpha$  3 chain mAb and  $\alpha$  5 chain mAb staining are absent in male samples and have a discontinuous distribution in female samples. The findings can be seen in 60-70% of the samples. In autosomal recessive samples staining of  $\alpha$  5 chain mAb is seen on Bowman's capsule and collecting ducts BM while  $\alpha$  3 chain mAb is negative.

Staining with  $\alpha$  3 chain mAb is not normally found in the skin while  $\alpha$  5 chain mAb stains the epidermal basement membrane. This staining is usually lacking in X-linked male samples while female samples exhibit a segmental distribution of the antigen. In autosomal recessive samples, staining of  $\alpha$  5 chain mAb is normal on the epidermal basement membrane.

This kit is for research use only. Not for use in diagnostic procedures.

## DOCUMENTATION

The following staining patterns will be obtained on the respective basement membranes.

	<b>Normal</b>		<b>Alport dominant</b>		<b>Alport recessive</b>	
	<b>kidney</b>	<b>skin</b>	<b>kidney</b>	<b>skin</b>	<b>kidney</b>	<b>skin</b>
$\alpha$ 1 chain mAb	+	+	+	+	+	+
$\alpha$ 3 chain mAb	+	-	-	-	-	-
$\alpha$ 5 chain mAb	+	+	-	-	-*	+

\*  $\alpha$  5 chain mAb staining is seen on Bowman's capsule and collecting ducts basement membrane.

## STORAGE

Store antibodies at 4°C, and Glycine/Urea Solution at -20°C.

## FOR RESEARCH USE ONLY

**KAMIYA BIOMEDICAL COMPANY**  
 12779 Gateway Drive, Seattle WA 98168  
 Tel: (206) 575-8068 Fax: (206) 575-8094  
 Email: LifeScience@k-assay.com  
 www.k-assay.com