

## Histological Evaluation of Renal Fibrosis in Mice

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### 1. Summary

This protocol describes a protocol to evaluate histological fibrosis in mouse kidney.

Reagent/Material	Quantity Required	Vendor	Stock Number
Picrosirius Red Stain Kit	1	Polysciences Inc	24901-250
Anti Collagen $\alpha$ antibody	1	Abcam plc.,	ab6586
70%, 90% and 100% Ethanol			
PBS, pH7.4			
Xylene			
10 % formalin			
Coverslips			
Staining Rack	2		

### 2. Reagents and Materials:

### 3. Tissue Sampling

The mice were anesthetized and kidneys were perfused with PBS via left ventricle. The kidneys were resected, fixed with 10% formalin, and the median 50% in each kidney was subjected to paraffin section processing.

### 4. Tissue stain

#### a. picrosiriusred stain

Collagen including collagen I and III were stained with the Picrosirius Red Stain kit (Polysciences Inc., PA, US) according to the manufacturers' protocol.<sup>1</sup>

1. Dip in Xylene 5 min x 2 times
2. Dip in 100 % EtOH 5 min x 2 times
3. Dip in 90 % EtOH 5 min
4. Dip in 80 % EtOH 5 min
5. Dip in 70 % EtOH 5 min
6. Dip in 50 % EtOH 5 min
7. PBS wash 5 min x 3 times
8. Solution A 2 min
9. PBS wash 5 min x 3 times
10. Solution B 60 min

11. Solution C 2 min
12. 70 % EtOH 45 sec
13. Dehydration, Cleaning, Mount

## **b. Collagen IV immunostain**

For Collagen IV staining, slides were placed on the Bond Max immunohistochemistry (IHC) stainer (Leica Biosystems., IL, US). Antibodies against primary collagen IV (Abcam plc., Cambridge, UK) <sup>2</sup> diluted 1:600 were applied to sections and incubated for one hour. All procedure was carried out at Translational Pathology Shared Resource at Vanderbilt University Medical Center.

## **5. Computational Quantification**

The Bond Refine Polymer detection system (Leica Biosystems.) was used for visualization. Region of interest (ROI) was manually defined as cortical region, which is combined cortex and outer stripe of outer medulla (OSOM). The Picrosirius red- (red) and collagen IV-positive areas (dark) in cortex were calculated using image analysis software (Digital Image Hub; Leica Biosystems.) with unified threshold respectively. The regional fibrosis index was estimated by the area percentage of the positive pixels.

$$\text{positive collagen 4} = \frac{\text{Area (Dark)}}{\text{Total Area}} \times 100\%$$

$$\text{positive picrosirius red} = \frac{\text{Area (Red)}}{\text{Total Area}} \times 100\%$$

Analyses were performed for 6 - 7 sections of each kidney (12 – 14 sections per mouse).

## **6. References**

1. Katagiri D, Hamasaki Y, Doi K, *et al.* Interstitial renal fibrosis due to multiple cisplatin treatments is ameliorated by semicarbazide-sensitive amine oxidase inhibition. *Kidney Int* 2015; **89**: 374 - 385.
2. Hara S, Umeyama K, Yokoo T, *et al.* Diffuse glomerular nodular lesions in diabetic pigs carrying a dominant-negative mutant hepatocyte nuclear factor 1-alpha, an inheritant diabetic gene in humans. *PLoS One* 2014; **9**: e92219.