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## Histological Evaluation of Renal Fibrosis in Mice

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**Protocol status:** Working

**We use this protocol and it's working**

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**Keywords:** histological fibrosis, nephropathy, histological fibrosis in mouse kidney, histological evaluation of renal fibrosis, diffuse glomerular nodular lesions in diabetic pig, renal fibrosis, interstitial renal fibrosis due to multiple cisplatin treatment, interstitial renal fibrosis, histological fibrosis, negative mutant hepatocyte nuclear factor, diabetic pig, mouse kidney, inheritant diabetic gene in human, inheritant diabetic gene, diabetic complication, glomerular nodular lesion, kidney int

## Abstract

### Summary

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

### Diabetic Complication:



Nephropathy

## 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.



## Materials

### MATERIALS

☒ Picosirius Red Stain Kit (1) **Polysciences, Inc. Catalog #24901-250**

☒ Anti Collagen antibody (1) **Abcam Catalog #ab6586**

☒ 70% 90% and 100% Ethanol

☒ PBS pH7.4

☒ Xylene

☒ 10 % formalin

☒ Coverslips

☒ Staining Rack (2)

### Note:

**Abcam ([RRID:SCR\\_012931](#))**

**Abcam Cat# [ab6586](#), [RRID:AB\\_305584](#)**

## Before start

### 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.

## 1 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.

## 2 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).