Aug 12, 2019

Histological Evaluation of Renal Fibrosis in Mice

Daisuke Katagiri[1], Takamune Takahashi[1]

[1]Vanderbilt University

1 Works for me dx.doi.org/10.17504/protocols.io.3gygjxw

Diabetic Complications Consortium
Tech. support email: rmcindoe@augusta.edu

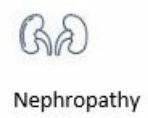
Lili Liang

ABSTRACT

Summary

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

Diabetic Complication:



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.*

EXTERNAL LINK

https://www.diacomp.org/shared/document.aspx?id=302&docType=Protocol

MATERIALS

NAME	CATALOG #	VENDOR
Picrosirius Red Stain Kit (1)	24901-250	Polysciences Inc
Anti Collagen antibody (1)	ab6586	Abcam
70% 90% and 100% Ethanol		
PBS pH7.4		
Xylene		
10 % formalin		
Coverslips		
Staining Rack (2)		

MATERIALS TEXT

Note:

Abcam (RRID:SCR_012931)

Abcam Cat# ab6586, RRID:AB_305584

BEFORE STARTING

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.[1]

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) [2] 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.

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

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

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