

## Klotho IHC for kidneys (Eva)

AbCam 154163

- Melt paraffin 1 hour 55-58 degrees
- Deparaffin (xylene 5 min x 2)
- Rehydrate in graded alcohol  
(100% alcohol 3 min → 95% alcohol 3 min → 70% alcohol 3 min)
- wash PBS 3 min
- Antigen retrieval : 20 minutes in sodium citrate buffer pH 6 in steamer (97 degrees), then cool 30 min at room temp

Sodium Citrate buffer: 2.05 mL of 1 M sodium citrate, 450 uL of 1 M citric acid in 250 mL total volume (water that is microwaved for 1.5 minutes)

-Wash PBS 3 min

- 3% H2O2 in methanol 20 minutes (25 mL 30% H2O2 in 225 mL Methanol)
- Wash PBS 3 min
- Block slides 1 hour (10% HiFBS in PBS)
- Primary Antibody 5 ucg/mL (10% HiFBS in PBS) → overnight in 4 degrees in humidified chamber
- Wash 3x TBST 5 min each
- Secondary antibody → anti-rabbit HRP (santa cruz) 1:600 in (10% HiFBS in PBS) for 1 hour at room temperature
- Wash 3x TBST 5 min each
- Develop with DAB (5 mL H2O with 4 drops Dab (vector) , 2 drops buffer, 2 drops H2O2)
- Fast Green Stain (FG for 1 minute → 1% acetic acid 30 sec → dip H2O)
- Dehydrate (2 min each of 70% alcohol → 95% alcohol → 100% alcohol → xylene)
- Coverslip