IHC protocol for mice Anti-phosphoSMAD3
(rabbit polyclonal ab-51177 Abcam)

Day 1
Paraffin Sections
1. Heat paraffin slides for at least 1hr at 55°C to melt wax
2. Deparaffinize slides in 2 changes of xylene x 5min each
3. Rehydrate in graded alcohol 100% ➔ 95% ➔ 70%, 3min each
4. PBS wash 5min
5. Antigen Retrieval 1: Incubate with 90% methanol+10%DMSO 20min RT. Wash PBS 3min
6. Endogenous peroxidase blocking slides in 0.3% H2O2 in methanol(200mL methanol+2mL H2O2): 20min RT
7. Antigen Retrieval 2: Citrate buffer pH=6.0 -> 20 min water bath 95-97°C. Wait 30 min
8. Endogenous unspecific protein blocking in TNB 30 min RT
Do Not Wash
9. Primary AB- 1:100 anti-pSMAD3 in TNB 4C overnight

Day 2
10. TBST wash 3x 5min each
11. Secondary AB-1:300 anti-rabbit Biotinylated in TNB 1HR RT
12. TBST wash 3x 5min each
13. SA-HRP (diluted 1:100 in TNB ) 30min RT
14. TBST wash 3x 5min each
15. Biotinyl Tyramide working solution 10min RT
16. TBST wash 3x 5min each
17. SA-HRP (diluted 1:100 in TNB) 1hr RT
18. TBST wash 3x 5min each
19. DAB Detection
1. 5ml ddH2O +2 drops buffer + 4 drops DAB + 2 drops H2O2
20. Stop reaction in ddH2O
21. Counterstain with Fastgreen 1 min ➔ Acetic Acid 30 sec ➔ ddH2O
22. Dehydrate in graded alcohol 70% ➔ 95% ➔ 100% ➔ Xylene
23. Coverslip

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