A1R-ABC staining:

1, perfusing rat with 4% paraformaldehyde in phosphat-bufferedsaline

2, Tissues were excised and fixation in the same fixative at 4℃ for 2hr or overnight.

3, Move the tissue into 30% sucrose in phosphate-buffered saline

4, Dry-ice frozen sections to be used14-20 μm of thickness and cut on a cryostat microtome

5, Stainig (全ての反応は切片を完全に広げて沈んでから振蕩する).

0.1%Triton X-100(PBS) 10min

PBS 5min x 3

1%NGS(100μl nomal Goat serum in 10ml PBS) 1hr

First antibody 1/1000 in 0.02%Triton (A1R 1μl＋0.1%Triton 200μl ＋799 μl 1%NGS) 1hr O/N
PBS 5min x 3

Second antibody 1/200 boitin-aR (in 1%NGS) 40min

PBS 5min x 3

standard kit C solution 45 min

PBS 5min x 3

0.1M Tris buffer (pH7.2)

0.1%DAB ＋ 0.02%H2O2 7min

0.1M PB (phosphate buffer)

Stick on coated slide glass 3hr

dehydrated through ethanol series and xylen

From Ren