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ImmPRESS Polymer Detection Following BaseScope In Situ Hybridization

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We use this protocol and it's working

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Abstract

Protocol for performing ImmPRESS immunostaining with alkaline phosphatase labeling after BaseScope In Situ Hybridization mouse brain sections.

Materials

Reagents: ImmPRESS®-AP Horse Anti-Rabbit IgG Polymer Detection Kit (MP-5401; Vector Labs) Vector® Blue

Alkaline Phosphatase Substrate (SK-5300; Vector Labs)

Day 1

1 Complete BaseScope in situ hybridization using 35um mouse brain sections. Do not dry slides. For BaseScope protocol see : (**dx.doi.org/10.17504/protocols.io.5qpvo364zv4o/v1**)

2 Wash slides 2 times in PBS at room temp 5 minutes each wash

3 Block sections in 0.4% Bovine Serum Albumin (BSA), 10% horse serum, 0.3% triton X-100 for 1 hour at room temp

4 Incubate sections in rabbit anti TH (1:500) in 0.4% BSA, 5% horse serum and 0.1% tritonX-100 overnight at 4 degrees C

Day 2

5 Wash slides in PBS at room temp (3 times 5 minutes each wash)

6 Incubate slides in secondary antibody (ImmPRESS-AP Polymer Reagent) at room temp for 2 hours.

7 Wash slides in PBS at room temp (3 times 5 minutes each wash)

8 1. Prepare the Alkaline Phosphatase Substrate

a. Make 2.5mL 100mM Tris-HCl, pH 8.2
b. Add 1 drop of Reagent 1
c. Add 1 drop of Reagent 2
d. Add 1 drop of Reagent 3
e. Mix well – use immediately

9 Incubate sections in alkaline phosphatase substrate for 5 minutes

10 Wash slides in PBS (2 times 5 min each wash)

11 Dry slides at 60°C for ≥15min until they are completely dry

12 Coverslip slides using VectaMount

13 Store slides at room temp

Protocol references

Preparation of Free Floating Coronal Mouse Brain Sections : **dx.doi.org/10.17504/protocols.io.dm6gp3pb8vzp/v1**

BaseScope In Situ Hybridization: (**dx.doi.org/10.17504/protocols.io.5qpvo364zv4o/v1**)