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FISH and antibody staining

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1 Works for me

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ABSTRACT

55-65°C anti-dig-POD 4°C anti-dig-AP

PROTOCOL CITATION

2020. FISH and antibody staining. **protocols.io**
<https://protocols.io/view/fish-and-antibody-staining-bmurk6v6>

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ABSTRACT

55-65°C anti-dig-POD 4°C anti-dig-AP

BEFORE STARTING

1. 1.5ml EP egg water 4% PFA 2. PBT 3. 1ml MetOH shake for 5min×3 4. -20°C

Day1

1 75%MetOH+25%PBT 1ml shake for 5min 00:05:00

2 50%MetOH+50%PBT 5min 00:05:00

3 25%MetOH+75%PBT 5min 00:05:00

4 100%PBT 5min 1/4 00:05:00



6 100%PBT 5min 3/4 🕒 00:05:00

7 100%PBT 5min 4/4 🕒 00:05:00

8  Proteinase K: PBT = 1 : 2000 ( 5µg/ml  5min  00:05:00

9 4%PFA 20min



00:20:00

10 1xPT 20min 1/3 00:20:00

11 1xPT 20min 2/3  00:20:00

12 1xPT 20min 3/3  00:20:00

13 1xPBT 5min 1/4 00:05:00

14 1xPBT 5min 2/4 00:05:00

15 1xPBT 5min 3/4 🕒 00:05:00

16 1xPBT 5min 4/4 00:05:00

17 500µl Hybe buffer 65°C 2–5h (63-68.5°C), 03:00:00

18 6µl probe+200µl Hybe buffer 65°Covernight 65 °C

Day2

19 -20°C 700-800µl 750 µl 65 °C



65°C 29

20 Hybe buffer 10min 00:10:00

21 75% Hybe buffer+25% 2×SSCT 10min 00:10:00

22 50%Hybe buffer+50% 2×SSCT 10min 00:10:00

23 25%Hybe buffer+75% 2×SSCT 10min 00:10:00

24 2×SSCT 10min 00:10:00

25 0.2×SSCT 15min 1/4 00:15:00

26 0.2×SSCT 15min 2/4 00:15:00

27 0.2×SSCT 15min 3/4 00:15:00

28 0.2×SSCT 15min 4/4 00:15:00

29 75% 0.2× SSCT+25% MABt 5min ⌚ 00:05:00 🌡 Room temperature 🧴 1 mL

30 50% 0.2× SSCT+50% MABt 5min ⌚ 00:05:00

31 25% 0.2× SSCT+75% MABt 5min ⌚ 00:05:00

32 MABt 5min ⌚ 00:05:00

33 1×blocking 500μl 700μl 2h RT ⌚ 02:00:00
🌡 Room temperature

34 Anti-Dig-POD 1:1000 200μl 4°C 4 °C



in situ Anti-Dig-POD

Day3

35

36 1×MABt 15min 1/8 ⌚ 00:15:00

37 1×MABt 15min 2/8 ⌚ 00:15:00

38 1×MABt 15min 3/8 ⌚ 00:15:00

39 1×MABt 15min 4/8 ⌚ 00:15:00

40 1×MABt 15min 5/8 ⌚ 00:15:00

41 1×MABt 15min 6/8 🕒 00:15:00

42 1×MABt 15min 7/8 🕒 00:15:00

43 1×MABt 15min 8/8 🕒 00:15:00

44 PT 20min 1/3 🕒 00:20:00

45 PT 20min 2/3 🕒 00:20:00

46 PT 20min 3/3 🕒 00:20:00

47 PBS 5min 1/2 🕒 00:05:00

48 PBS 5min 2/2 🕒 00:05:00

49 100µl Cys TSA+49µl magnification buffer Green/Red 🔥 Room temperature 50 µl

 

Day4

50 

51 PT 20min 1/8 🕒 00:20:00

52 PT 20min 2/8 🕒 00:20:00

53 PT 20min 3/8 🕒 00:20:00

54 PT 20min 4/8 🕒 00:20:00

55 PT 20min 5/8 🕒 00:20:00

56 PT 20min 6/8 🕒 00:20:00

57 PT 20min 7/8 🕒 00:20:00

58 PT 20min 8/8 🕒 00:20:00

59 +blocking PBTN 500μl 4°C 2h ⚡ 4 °C 🕒 02:00:00 🧴 500 μl

60 +PBTN=1:1000 100μl 4°C Dendra2 Rabbit-anti-Dendra2 Kaeda Rabbit-anti-kaeda Mcherry Mouse-anti-mcherry CFP/GFP Ab6658 goat A1122 rabbit Sc9996 mouse DsRed Mouse-anti-DsRed
🧴 100 μl ⚡ 4 °C

Day5

61 4°C

62 PT 20min 1/8 🕒 00:20:00

63 PT 20min 2/8 🕒 00:20:00

64 PT 20min 3/8 🕒 00:20:00

65 PT 20min 4/8 🕒 00:20:00

66 PT 20min 5/8 🕒 00:20:00

67 PT 20min 6/8 🕒 00:20:00

68 PT 20min 7/8 🕒 00:20:00

69 PT 20min 8/8 PBTN blocking 🕒 00:20:00

70 in PBTN 1:2000 200 μl 4 °C donkey-goat 633 donkey-rabbit 488 donkey-rabbit 633
200 μl 4 °C

Day 6

71

72 PT 20min 1/8 🕒 00:20:00

73 PT 20min 2/8 🕒 00:20:00

74 PT 20min 3/8 🕒 00:20:00

75 PT 20min 4/8 🕒 00:20:00

76 PT 20min 5/8 🕒 00:20:00

77 PT 20min 6/8 🕒 00:20:00

78 PT 20min 7/8 🕒 00:20:00

79 PT 20min 8/8 ⌚ 00:20:00

80 1×PT4°C