Sep 30, 2020

in situ hybridization

Forked from FISH and antibody staining

[1]

[1]

1 Works for me This protocol is published without a DOI.

Zhang Xue

PROTOCOL CITATION

2020. in situ hybridization. **protocols.io** https://protocols.io/view/in-situ-hybridization-bmvzk676

FORK FROM

Forked from FISH and antibody staining,

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CREATED

Sep 30, 2020

LAST MODIFIED

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PROTOCOL INTEGER ID

42649

BEFORE STARTING

1. 1.5ml EPegg water4% PFA 2. PBT2 3. 1ml MetOHshake 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**

PK

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5 100%PBT 5min 2/4 **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 : 1000 (10μg/ml24h24h5min36h10min48h15min60h20min72h30min
00:00:00 **00:10:00** **00:15:00** **00:20:00** **00:30:00**

9 4%PFA 30min

PFA

00:30:00

10 1×PBT 5min 1/4 **00:05:00**

11 1×PBT 5min 2/4 **00:05:00**

12 1×PBT 5min 3/4 **00:05:00**

13 1×PBT 5min 4/4 **00:05:00**

14 500μl Hybe buffer68.5°C2–5h **68.5 °C** **03:00:00**

15 6μl probe+200μl Hybe buffer68.5°C overnight **68.5 °C**

Day2

16 -20°C700-800μl **750 μl** **68.5 °C**

68.5°C29

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17 Hybe buffer 10min **00:10:00**

18 75% Hybe buffer+25% 2×SSCT 10min **00:10:00**

19 50%Hybe buffer+50% 2×SSCT 10min **00:10:00**

20 25%Hybe buffer+75% 2×SSCT 10min **00:10:00**

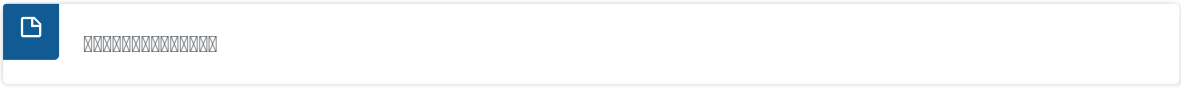
21 2×SSCT 10min **00:10:00**

22 0.2×SSCT 15min 1/4 **00:15:00**

23 0.2×SSCT 15min 2/4 **00:15:00**

24 0.2×SSCT 15min 3/4 **00:15:00**

25 0.2×SSCT 15min 4/4 **00:15:00**



26 75% 0.2× SSCT+25% MABt 5min **00:05:00** **Room temperature** **1 mL**

27 50% 0.2× SSCT+50%MABt 5min **00:05:00**

28 25%0.2× SSCT+75%MABt 5min **00:05:00**

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29 MABt 5min **00:05:00**

30 1×blocking 500µl□□□□700µl□2h□RT **02:00:00**

Room temperature **500 µl**

31 Anti-Dig-AP 1:2000□200µl□4°C□□□□□□□□ **4 °C** **200 µl**

□□□FISH□□□□□□Anti-Dig-AP

Day3

32 □□□□□□□□4°C□□

33 1×MABt 15min 1/8 **00:15:00**

34 1×MABt 15min 2/8 **00:15:00**

35 1×MABt 15min 3/8 **00:15:00**

36 1×MABt 15min 4/8 **00:15:00**

37 1×MABt 15min 5/8 **00:15:00**

38 1×MABt 15min 6/8 **00:15:00**

39 1×MABt 15min 7/8 **00:15:00**

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40 1×MABt 15min 8/8□□□NTMT□□□□□□□□□□3.5ml□ **00:15:00**

20ml	NTMT
2ml	1M pH9.5 Tris-HCl
2ml	1M NaCl
0.5ml	10% Tween
15.5ml	ddH2O

41 Equilibrate with NTMT□5min 1/3 **00:05:00**

42 □□□24□□□NTMT 5min 2/3 **00:05:00**

□□□□□BCIP in NTMT□1:50□500µl□□□

43 NTMT 5min 3/3 **00:05:00**

44 □□□BCIP in NTMT□1:50□500µl□□37°C□□□□□15min□□□□□BF□□□□□□ **500 µl** **37 °C** **00:30:00**

□□

45 1ml stop solution □□□□□□□□□□□□□□□□□□

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