



Sep 30, 2020

TUNEL

Forked from [FISH and antibody staining](#)

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1 Works for me

This protocol is published without a DOI.

Zhang Xue



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PROTOCOL CITATION

2020. TUNEL. [protocols.io](https://protocols.io/view/tunel-bmv7k69n)
<https://protocols.io/view/tunel-bmv7k69n>

FORK FROM

Forked from [FISH and antibody staining](#), 1

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CREATED

Sep 30, 2020

LAST MODIFIED

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PROTOCOL INTEGER ID

42655

BEFORE STARTING

1. 1.5ml EP tube egg water 4% PFA 4°C
2. PBT 5min×3
3. 100% MetOH 5min×3 -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

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 加蛋白酶 K: PBT = 1 : 2000 (加 5μg/ml 5min) 00:05:00

9 100%PBT 5min 1/2 00:05:00

10 100%PBT 5min 2/2 🕒 00:05:00

11 样品: 1:2, -20°C 30min  -20 °C  00:30:00

12 浸4%PFA 20min

[illegible]

00:20:00

13 1xPBT 5min 1/3 00:05:00

14 1xPBT 5min 2/3 00:05:00

15 1xPBT 5min 3/3  00:05:00

16 +45μLequilibration bufferRhoche37°C 37 °C 02:00:00



XXXXXXXXXX

17 +5µL Rhoche 37°C 1 1.5h 37 °C 01:30:00

18 2×SSCT 37°C 20min 1/2 37 °C 00:20:00

19 2×SSCT 37°C 20min 2/2 37 °C 00:20:00

20 2×SSCT 20min Room temperature 00:20:00

21 1×PBT 5min 1/3 00:05:00

22 1×PBT 5min 2/3 00:05:00

23 1×PBT 5min 3/3 00:05:00

24 +blocking PBTN 500µl 4°C 2h 4 °C 02:00:00 500 µl

25 +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

Day2

26 4°C

27 PT 20min 1/8 00:20:00

28 PT 20min 2/8 00:20:00

29 PT 20min 3/8 🕒 00:20:00

30 PT 20min 4/8 🕒 00:20:00

31 PT 20min 5/8 🕒 00:20:00

32 PT 20min 6/8 🕒 00:20:00

33 PT 20min 7/8 🕒 00:20:00

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

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

Day3

36

37 PT 20min 1/8 🕒 00:20:00

38 PT 20min 2/8 🕒 00:20:00

39 PT 20min 3/8 🕒 00:20:00

40 PT 20min 4/8 🕒 00:20:00

41 PT 20min 5/8 🕒 00:20:00

42 PT 20min 6/8 🕒 00:20:00

43 PT 20min 7/8 🕒 00:20:00

44 PT 20min 8/8 🕒 00:20:00

45 1×PT 4°C