BrdU-ISH double labeling method

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Here I wrote non-fluorescent and fluorescent protocols to double-label BrdU and ISH. These methods are to be combined with our protocol for normal ISH. BrdU is a Thymidine analogue and is now widely used to label dividing cell populations. For example, in the field of developmental neuroscience, it has been used in place of 3H-thymidine for neuronal birthdating. That is, particular types of neurons are often generated at certain developmental timepoints and stop dividing, and thus can be labeled by BrdU by simply injecting the pregnant animals with BrdU solution at those timepoints. BrdU labeling is also used to identify the newly generated neurons in the adult brains.

<Reagents>

BrdU solution (20 mg/ml) BrdU (sigma#B5002) in 0.007 N NaOH, 0.9% NaCl
PBST PBS with 0.2 % TritonX100
Anti-BrdU Abcam: BrdU antibody [BU1/75 (ICR1)]
Elite ABC kit Vectastain: PK-6100
Streptavidin-Cy3 Jackson ImmunoResearch laboratory: #016-160-084
TNT TS7.5, 0.05% Tween20

<BrdU labeling>

Inject BrdU solution into pregnant animals (50 mg/kg, i.p.).
Fix the offsprings at desired timepoints with 4% paraformaldehyde/0.1M PB by cardiac perfusion. Dissect out the brain and cut sections.

BrdU-ISH method (DAB-NBT/BCIP)

Fix in 4% PFA /0.1M PB at 4℃ o/n

0.1M PB 10min x2
0.75% Glycine/0.1M PB 15min x2
0.3% Triton X100/0.1M PB 20min
0.1M PB 5min
ProK at 37℃ 30min
acetylation10min
0.1M PB 10min x2
Prehybridization at 60℃1hr
Hybridization at 60℃ o/n

Wash 2xSSC/FA/NLS at 60℃ 15min x2
RNaseA Buffer 5min
RNaseA(20ug/ml) at 37℃ 30min
2xSSC/NLS at 37℃ 15min x2
0.2xSSC/NLS at 37℃ 15min x2
TS7.5
Blocking 1%Blocking Reagent/TS7.5 30min
anti-DIG-AP (1/1000) in 1%Blocking at RT 2hr or at 4℃ o/n
Wash TNT 15min x3
TS9.5 15min
NBT/BCIP(1/50) in TS9.5 1hr
Wash PBS 10min x2

1.5N HCl at 37℃ 30min
Wash TNT 5min x2
Blocking 5% skim milk in PBST(SM/PBST) 20min
anti-BrdU(1/75) in SM/PBST at 4℃ o/n

Wash PBS 10min x3
anti-rat-biotin(1/500) in SM/PBST at RT 2hr or at 4℃ o/n
Wash TNT 10min x3
ABC(A:1/100, B:1/100, in TNT) 30min
Wash TNT 10min x3
DAB(H_2O_2:1/1000, DAB:1/100, in TNT) 10min
Wash PBS

BrdU-ISH method (Fluorescent)

Fix in 4% PFA /0.1M PB at 4℃ o/n

0.1M PB 10min x2
0.75% Glycine/0.1M PB 15min x2
0.3% Triton X100/0.1M PB 20min
0.1M PB 5min
ProK at 37℃ 30min
acetylation10min
0.1M PB 10min x2
Prehybridization at 60℃1hr
Hybridization at 60℃ o/n

Wash 2xSSC/FA/NLS at 60℃ 15min x2
RNaseA Buffer 5min
RNaseA(20ug/ml) at 37℃ 30min
2xSSC/NLS at 37℃ 15min x2
0.2xSSC/NLS at 37℃ 15min x2
TS7.5
Bloching 1%Blocking Reagent/TS7.5 30min
anti-DIG-HRP (1/2000) in 1%Blocking at RT 2hr or at 4℃ o/n
Wash TNT 15min x3
TSA-plus(1/50) 30min
Wash TNT 10min x3

1.5N HCl at 37℃ 30min
Wash TNT 5min x2
Blocking 5% skim milk in PBST(SM/PBST) 20min
anti-BrdU(1/75) in SM/PBST at 4℃ o/n

Wash PBS 10min x3
anti-rat-biotin(1/500) in SM/PBST at RT 2hr or at 4℃ o/n
Wash TNT 10min x3
ABC(A:1/100, B:1/100, in TNT) 30min
Wash TNT 10min x3
anti-streptavidin-Cy3(1/500), anti-DNP-Alexa488(1/500) in SM/PBST at RT 2hr or at 4℃ o/n
Wash TNT x3