

Non-radioactive *in situ* hybridization on paraffin sections using DIG-labeled miRCURY™ detection probes

Protocol prepared by Dr. Wigard Kloosterman, the Plasterk Group, Hubrecht Laboratory, Utrecht, The Netherlands

Deparaffinize the sections

- Xylene 3x 5min
- Ethanol 100% 2x 5min
- Ethanol 70% 5min
- Ethanol 50% 5min
- Ethanol 25% 5min
- DEPC 1x 1min

Deplete the sections

- 2 x 5 min PBS
- 5 min Prot.K at 10 µg/mL at 37°C (add Prot.K 20mg/mL to warm Prot.K buffer 20 min before incubation)
- 30 sec 0.2% Glycine in PBS
- 2 x 30 sec PBS
- Fix sections for 10 min in 4% PFA*
- Rinse slides 2 x in PBS

Prehybridization

- 2h-in hybridization buffer (50%Formamide, 5xSSC, 0.1%Tween, 9.2 mM citric acid for adjustment to pH6, 50 µg/mL heparin, 500 µg/mL yeast RNA) in a humidified chamber (50% formamide, 5xSSC). Use DAKO Pen.

Hybridization (hybridization temperature=T_m probe -21°C)

- Dilute probe to 20 nM in hybridization buffer
- Add 200 µL hybridization mix per slide
- Hybridize slides overnight covered with Nescofilm in a humidified chamber

Stringency Wash

- Rinse in 2 x SSC at hybridization temperature
- 3 times 30 min in 50% formamide, 2 x SSC at hybridization temperature
- 5 x 5 min in PBST at RT

Immunological Detection

- 1 h block in blocking buffer (2% sheep serum, 2mg/mL BSA in PBST) at RT
- o/n antibody incubation (1:2000 anti-DIG-AP Fab fragments in blockingbuffer) in a humidified chamber at 4°C
- Wash 5-7 times 5 min in PBST
- Wash 3 times 5 min in AP buffer



Colour reaction (RT, darkness)

- light sensitive colour reaction (NBT/BCIP) 1h-48h (400 µL/slide) in a humidified chamber
- Wash slides 3x 5 min in PBST
- Mount in aqueous mounting medium (glycerol) or dehydrate and mount in Entellan.

Buffers

Colour solution (Light sensitive)

- 45 µL 75mg/mL NBT (in 70% dimethylformamide)
- 35 µL 50mg/mL BCIP-phosphate (in 100% dimethylformamide)
- 2.4 mg Levamisole in 10 mL AP buffer.

AP buffer (100 mM Tris HCl pH9.5, 50 mM MgCl₂, 100 mM NaCl, 0.1% Tween 20)

- 100 mL Tris (100mM) 12.1g/L
- 20 mL 5M NaCl (100mM) 5.84g/L
- 5 mL 1M MgCl₂ (5mM)
- 700 mL sterile H₂O, pH 9.5 and fill up to 1L

*Please note: For optimal fixation it may be critical to use fresh formaldehyde solutions. Fresh 4% solutions can be made from 16%, methanol free, formaldehyde or from solid paraformaldehyde (4% w/v).

For preparation of buffers please refer to :

Molecular cloning : a laboratory manual / Sambrook, Joseph; Russell, David W. --
3rd ed. -- New York: Cold Spring Harbor Laboratory, 2001.

DIG: DIG is licensed from Roche Diagnostics GmbH.

"This protocol has been developed by a third party and not by Exiqon A/S or group companies ("EXIQON").

Thus, EXIQON cannot and will not warrant, represent or in any other way guarantee that the protocol and its content comply with your needs or expectations. EXIQON excludes all liability for any illegality arising from error, omission or inaccuracy in the protocol and takes no responsibility for the protocol or otherwise.

To the extent permitted by law, EXIQON excludes all liability in contract, tort (including negligence), breach of statutory duty or otherwise for any costs, losses, claims, damages, expenses or proceedings (including special, incidental or consequential loss or damage, loss of profits and wasted management time) incurred or suffered by you arising directly or indirectly in connection with the protocol and its content, including any loss, damage or expense arising from, but not limited to, any defect, error, imperfection, fault, mistake or inaccuracy with the protocol and its content. Any dispute relating to the protocol involving EXIQON shall be governed by Danish law."

