Tissue Microarray (TMA)

ISH (*In situ* hybridisation non-radio-active)

GENERAL NOTE

This Product Application Information is based on standard application protocols, modified on the basis of our own lab experience.
As handling in numerous labs is quite different oligene can not guarantee for the results using this protocol, as well as for mistakes in the protocol. As it does not contain any safety instruction, the operator is responsible to inform about and to follow the safety instructions of the supplier of any material involved.

HANDLING NOTE

Be aware, that increased mechanical stress during TMA slide treatment may cause dislodging of spots! All wash solutions and reagents should be applied gently to the slide.

Protocol: *In situ* hybridisation non-radio-active

Pre-treatment of TMA slide:

- Incubate the slide 30´at 70°C or over night at 60°C
- Remove paraffin wax carefully (standard protocols of descending alcohol sequence) and rehydrate

deparaffination

- 3 x 10 min Xylol
- 2 x 5 min. 100% Ethanol (RT)
- 5 min. 96% Ethanol (add DEPC-H_2O)
- 5 min. 90% Ethanol (add DEPC- H_2O)
- 5 min. 70% Ethanol (add DEPC- H_2O)
- 5 min. 50% Ethanol (add DEPC- H_2O)
- 3 x 3 min. PBS/DEPC (RT)

Pre-hybridisation (Rnase free)

- 10 min. 4% PFA/PBS (RT)
- 2 x 3 min. PBS (RT)
- 10 min. 0,2M HCL (RT)
- 5 min. PBS (RT)
- 10 min. 1 µg/ml Proteinase K / 0,1M Tris-HCl, pH 8,0 (RT / 37°C)
- 2 x 5 min. PBS (RT)
- 30 min. acetylate with 0,1M Triethanolamin-HCl, pH 8,0 (control pH)
- add directly before use 5 mM Acetanhydrid (RT)
- 3 x 5 min. PBS (RT)

dehydrogenation

- in 30% Ethanol (add DEPC- H_2O)
- 1 min 70% Ethanol (add DEPC- H_2O)
- 1 min 90% Ethanol (add DEPC- H_2O)
- 1 min 100% Ethanol
- dry slide 1-2 h on clean blotting paper, label and sort in a hybridisation chamber

For Research Use Only

Not intended for use in diagnostic or therapeutic procedures.

oligene GmbH, Schumannstraße 20/21, 10117 Berlin , Germany · Telefon: +49(030)450 578 358, Fax: 450 578 919, e-mail: sales@oligene.com

Hybridisation (Rnase free)

- prepare wet chamber with 5 x SSC/50% Formamid
- dilute probe in Formamid 1 : 2; final concentration for ISH 2-4ng/µl
- denature probe 3 min. at 96°C
- rapid on ice; short spin down
- add Denhardt´s and probe to Hybridisation buffer
- add 25 µl on the slide
- apply cover slip, avoid air bubbles
- envelop wet chamber in cling film
- hybridisation 14-16h (over night) at 50 – 65°C (has to be tested)

Post-hybridisation (not Rnase free)

- detach cover slips in 5 x SSC (RT)
- 5 min. 5 x SSC (RT)
- 5 min. STE Buffer (RT)
- 60 min. 50µg/ml RNase A in STE Buffer (37°C) (1ml per OT)
- 5 min. STE Buffer (RT)
- 5 min. 5 x SSC (RT)
- 30 min. 2 x SSC; 0,1 SDS } shaker — T ↑; under Hybr. Temp.
- 60 min. 0,2 SSC; 0,1 SDS }
- 15 min. 0,2 x SSC (RT)

Immune reaction (not Rnase free)

- 5 min. MabS Buffer (RT)
- 60 min. 2% blocking-reagent in MabS Buffer (RT)
- 30 min. wet chamber with reaction buffer (anti Dig A; 1:750/2% Blocking reagent/MabS Buffer (RT) ca. 400 – 500µl per slide
- 3x10 min. MabS Buffer (RT); shaker
- 5 min. reaction buffer/1mM Levamisol
- transfer slide in wet chamber with reaction buffer
- per slide 500µl substratum solution BM Purple
- incubate in the dark at RT for 2h, then over night (4°C – RT, has to be tested)
- stop reaction with PBS
- rinse with water
- air-dry and seal with an aqueous mounting medium

Storage

- Store at +4°C in the dark

Orders and Technical Information

Oligene GmbH
Schumannstr. 20/21
D-10117 Berlin
Germany

Fon +49.30.450578358
Fax +49.30.450578919
sales@oligene.com
www.oligene.com

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