Tissue Microarray (TMA)

FISH (Fluorescence *In Situ* Hybridisation)

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 FISH (Fluorescence *In Situ* Hybridisation)

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

1. deparaffination
2. 0,2N HCL; 20' at RT
3. A.dest; 3' at RT
4. 2xSSC pH; 3' at RT
5. 30% Na-bisulfit ; 15' at 45°C (50ml- cuvette in water bath)
6. A.dest.; 1' at RT
7. 2xSSC pH7; 2x 5' at RT

- Unmask if necessary (check datasheet of your application kit / antibody specifications)

Proteinase K- digestion (Rnase free)

Before digestion drip 10µl Propidium Iodid / Glycerin- mixture on the slide and apply the cover slip for microscope control with Cy2/FITC- filter (I-3/L-5)

digestion

- dilute prepared **Proteinase K** with 2xSSC 1:20 30' at 55°C
- **alternative:** per 1' 70%, 80%, 90%, absolute alcohol
- dry on heating plate at 45°C

microscopic control of digestion:

- cover slide with 2xSSC

→not ok: rinse with 2xSSC, continue digestion
→ok: 2xSSC pH7 2x5' at RT

- check by microscopic control if PI-colorimeter is removed

→not ok: continue washing
→ok: dry 30' at 37°C

For Research Use Only

Not intended for use in diagnostic or therapeutic procedures.

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Hybridisation (Rnase free)

- drip 10µl probe on the slide, cover air bubble free and glue airtight
- coat the rear side of the slide with oil (heat bridge) and put it into incubator

Program:

- Denaturation 5' at 73°C
- Hybridisation over night at 37°C

Next day:

- Heat the cuvette with 2xSSC/0,2-0,3% NP40-Buffer (73°C), water bath
- Take the slide out of the incubator and put it into the cuvette with 2xSSC/0,2-0,3%NP40-Puffer (RT)
- Remove the cover slip carefully
- incubate the slide 2' at 72- 73°C
- rinse in TBS
- dry the slides in the dark (i.e. incubator 30' at 37°C)
- counterstain with 10µl DAPI II (nucleusfluorochrom/filter A)
- cover with cover slip and seal with Eukitt

Storage

- Store at +4°C in the dark

Orders and Technical Information

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