Tissue Microarray (TMA)

APPAP (Alkaline Phosphatase Anti-Alkaline Phosphatase)

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 APPAP (Alkaline Phosphatase Anti-Alkaline Phosphatase)

Pre-treatment of TMA slide:

- Incubate the slide over night at 37-58°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)
- 2 x 5 min. 96% Ethanol (add DEPC-H_2O)
- 2 x 5 min. 70% Ethanol (add DEPC- H_2O)
- Aqua dest.

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

implementation

- incubate slide 5 min. at RT in TBS
- swab excrescent wetness and cover the test area with optimal diluted prime antibody
- incubate 30 min. at RT in wet chamber
- wash 3 x 5 min. with TBS
- swab excrescent wetness and cover the test area with optimal diluted secondary antibody
- incubate 30 min. at RT in wet chamber
- wash 3 x 5 min. with TBS
- swab excrescent wetness and cover the test area with optimal diluted APAAP complex
- incubate 30 min. at RT in wet chamber
- wash 3 x 5 min. with TBS
- apply substrates (i.e. Fast Red or Fuchsine)
- incubate 10 min.
- rinse slide with H_2O
- nucleus staining with Hämatoxylin according to Mayer 5 min.
- blue in warm tap water for 5 min.
- seal with an aqueous mounting medium (i.e. glycerine-gelatine)

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

double APAAP (to increase the sensitivity)

step 1 – 10 noRTal APAAP method

- wash with TBS 3 x 5 min.
- swab excrescent wetness and cover the testarea with optimal diluted secondary antibody (repitition)
- incubate 10 min. at RT in a wet chamber
- wash 3 x 5 min. with TBS
- swab excrescent wetness and cover the testarea with optimal diluted APAAP complex (repitition)
- incubate 10 min. at RT in a wet chamber
- wash 3 x 5 min. with TBS
- apply substrate (i.e. Fast Red or Fuchsin)
- incubate 10 min.
- rinse slides with H_2O
- nucleus staining with Hämatoxylin according to Mayer 5 min.
- blue in warm tap water for 5 min.
- seal with an aqueous mounting medium (i.e. glycerin-gelatin)

notice:

- important: do not let slides run dry!
- decide the blocking options of background depending on used tissue and enzyme-substrate mixture
- using additional kits please follow the company advices

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