



## Protocol ♦ H&E Staining

The MMI H&E Staining Kit (PN 70302) is designed to quickly stain only a few samples, at the same time ensuring that the result is clean, clear and contamination free.

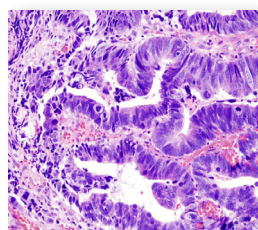
The staining solutions are supplied in MMI SafeStain ampoules, allowing quick handling without the need for pipetting or the preparation of staining jars.

The MMI SafeStain ampoule guarantees a uniform drop size, and ensures that the solutions remain contamination free. The staining solutions have been rigorously tested for the demanding needs of laser microdissection users. The clear staining is reproducibly achieved allowing a clear view of the samples together when viewed with the MMI Isolation Cap. Each kit contains 15 MMI SafeStain ampoules, designed for 30 - 60 staining sessions. Ampoules are designed for the staining of 2 - 4 slides.

The improved sample Lift-Off success rate is achieved due to quicker drying process. By choosing the MMI H&E Staining Kit you help to reduce the amount of water-hazardous stain waste and cut your laboratory running costs.

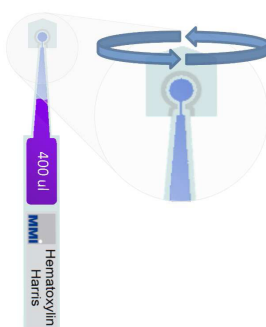
### Materials:

- > 98 % Xylene
- 100 % Anhydrous ethanol (reagent-grade)
- ddH<sub>2</sub>O (RNase-free)
- MMI H&E Staining Kit (PN 70302)



#### How to use the MMI SafeStain ampoule:

1. Hold the MMI SafeStain Ampoule upright as shown and tap the base twice on a table. This should cause the staining solution to be removed from the lid and be collected in the body of the ampoule. If solution still remains in the lid repeat the tap, until the solution has settled.
2. Now, open the MMI SafeStain Ampoule by twisting the lid off.
3. The stain can now be applied to the tissue by gently squeezing the ampoule. One drop is about 30  $\mu$ l in size and covers 50 - 100 mm<sup>2</sup> of tissue.



### Method:

This procedure is valid for all kinds of tissue sections which are 10  $\mu$ m or less. Thicker samples may require shorter staining times.

The entire procedure will take 10 minutes.

1. Tissue Preparation
  - 1.1. Prepare your tissue sections
  - 1.2. Fix and rehydrate tissue
    - Frozen tissue:
      1. Fix tissue in 75 % ethanol for 30 sec
      2. Dip in ddH<sub>2</sub>O for 30 sec
    - FFPE tissue
      1. Deparaffinize:
        - Dip in xylene 2 x for 3 min each
      2. Remove xylene and fixate:
        - Dip in 100 %, 95 %, 75 % ethanol for 30 sec each
        - Dip in ddH<sub>2</sub>O for 30 sec
2. Staining procedure
  - 2.1. Hematoxylin stain:  
Apply 1 drop of Hematoxylin stain per 50 - 100 mm<sup>2</sup> and wait 45 sec (ca. 5 drops per slide)
  - 2.2. Hematoxylin rinsing:  
Rinse Hematoxylin residues vigorously with warm (30 °C) ddH<sub>2</sub>O about 45 sec
  - 2.3. Eosin stain:  
Apply 2 - 3 drops of Eosin stain and wait 30 sec
  - 2.4. Eosin rinsing:  
Rinse again with cold ddH<sub>2</sub>O for 15 sec
3. Dehydrate and dry
  - Dip in 75 %, 95 %, 100 % ethanol for 30 sec each
  - Dip in xylene for 30 sec
  - Air dry for 30 - 60 sec

Your sample is now ready for laser microdissection.

#### Notes:

- To ensure that the sample remains contamination free we recommend using distilled water for washing steps.
- Instead of Ethanol, you can also use > 98 % Isopropanol.
- For best results always keep the MMI SafeStain Ampoules in the box when not used. Store at room temperature and protect from light as this can cause degradation of the stain. Avoid freezing.
- Note the "best before" date printed on the box for optimal stain quality.
- Do not reuse once opened ampoules because the stain starts to oxidize. Furthermore, contamination free status could not be guaranteed anymore.
- In case of overstaining, the samples should be washed using acid-alcohol to reduce the intensity.
- In case of understaining, the staining times for Hematoxylin should be increased.