

MMI Protocol

H&E Staining



Stain quick.
Stain clear.

Introduction

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.

Each kit contains 15 MMI SafeStain ampoules, designed for 30 - 60 staining sessions. Ampoules allow the staining of 2 - 4 slides. The improved sample Lift-Off success rate is achieved due to a 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 cost.

Materials

- > 98 % Xylene
- 100 % anhydrous ethanol (reagent-grade)
- ddH₂O (RNase-free)
- MMI H&E Staining Kit (PN 70302)



MMI H&E Staining Kit (PN 70302). Each kit contains 15 MMI SafeStain ampoule for up to 60 staining sessions.

Method

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



1. Tissue

- 1.1. Prepare your tissue sections.
- 1.2. Fix and rehydrate tissue.

Frozen tissue:

- a) Fix tissue in 75 % ethanol for 30 sec.
- b) Dip in ddH₂O for 30 sec.

FFPE tissue

- a) Deparaffinize:
 - Dip in xylene 2 x for 3 min each.
- b) Remove xylene and fixate:
 - Dip in 100 %, 95 %, 75 % ethanol for 30 sec each.
 - Dip in ddH₂O for 30 sec.



2. Staining procedure

- 2.1. Hematoxylin stain:
Apply 1 drop of Hematoxylin stain per 50 - mm² and wait 45 sec (ca. 5 drops per slide).
- 2.2. Hematoxylin rinsing:
Rinse Hematoxylin residues rigorously with warm (30 °C) ddH₂O 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 ddH₂O 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.

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 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 µl in size and covers 50 - 100 mm² of tissue.



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 the ampoules once opened to prevent stain oxidation and maintain a contamination-free status.
- 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.

