



Molecular Machines and Industries

H&E Staining Kit Plus

for Laser Microdissection and more

www.molecular-machines.com





Contact

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The material safety data sheets are available from the MMI
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Introduction

mmi H&E Staining Kit Plus is designed for users with a need to quickly stain only a few samples and need to ensure that the result is clean and 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 the solutions remain **contamination free**.

The staining solutions have been rigorously tested for the demanding needs of laser microdissection users. The **clear staining** they need is reproducibly achieved allowing a clear view of the samples together when viewed with the mmi IsolationCap.

Each kit contains 15 mmi SafeStain ampoules, designed for **30-60 staining sessions**. Ampoules are designed for the staining of 2-4 slides.

By choosing the mmi H&E Staining Kit Plus you help to **reduce the amount of** water hazardous stain waste and cut your laboratory running costs.



Kit content

The mmi H&E Staining Kit Plus includes:

15 hematoxylin mmi SafeStain ampoules contain:

Modified Harris hematoxylin nuclear stain
7 g/L Hematoxylin
without mercury

15 Eosin mmi SafeStain ampoules contain:

Eosin 2% (water soluble)

The following has to be provided by user:

Xylene

Propanol (recommended)

H₂O (distilled recommended)



Basic principles of H&E Staining

Hematoxylin is a widely used nuclear stain. Eosin is used solely to enhance the contrast of the stain.

The principle of H&E staining:

Hematoxylin stain acts as a cation and attaches to anions present in the sample, examples include the phosphate groups of DNA or lysine residues from chromatin located in the **nucleus** of the cell. Increasing the acidity to a pH-value above pH3, tissue sections change color from red to **blue**. Highly concentrated Hematoxylin stains like Gill 3 and Harris are progressive nuclear stains. They include more than 5 g/L of hematoxylin salt.

Eosin acts as an acid stain and interacts with the basic amino acid groups of proteins located in the **cytoplasm**. Eosin is a regressive stain that will quickly overstain the tissue. The tissue can then be destained with propanol until the required endpoint is reached. This results in a **pink-red** counter stain. Alcoholic eosin is used in cytology while aqueous eosin is preferred in histology.

If staining appears insufficient, the staining time of the progressive hematoxylin should be increased, and the destaining time of eosin should be decreased.



Procedure

This procedure is valid for all kinds of tissue sections which are 10µm or less. Thicker samples may require shorter staining times. The entire procedure will take 4 minutes.

If using fresh frozen tissue instead of FFPE, step 1 can be omitted.

1.) **Deparaffinize**

Dunk for 45 sec in xylene

2.) **Remove xylene and fixate**

Dunk for 45 sec in propanol

3.) **Hematoxylin stain:**

Apply 1 drop of Hematoxylin stain per 50-100 mm² and wait 45 sec. (ca. 5 drops per slide)

4.) **Hematoxylin rinsing**

Rinse hematoxylin residues vigorously with warm (30°C) water (distilled H₂O recommended) about 45 sec.

5.) **Eosin stain:**

Apply 2-3 drops of Eosin stain and wait 30 sec.

6.) **Eosin rinsing:**

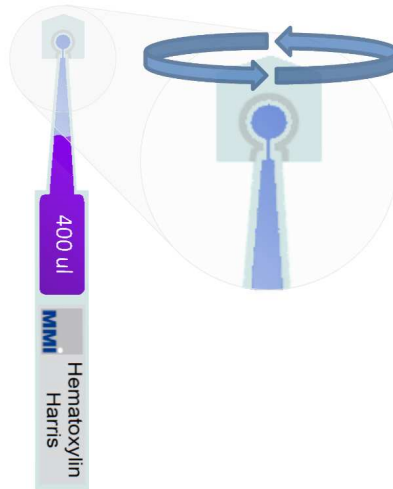
Rinse again with cold water (distilled H₂O recommended) for 15 sec.

7.) **Last wash**

Dip 45 sec in propanol and then 45 sec in xylene.



How to use the mmi SafeStain ampoule



Hold the mmi SafeStain Ampoule upright as shown above 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.

Now, open the mmi SafeStain Ampoule by twisting the lid off.

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.

Where possible use Propanol. It is more cost effective than ethanol and has been reported to produce better results for this procedure.

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.

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.