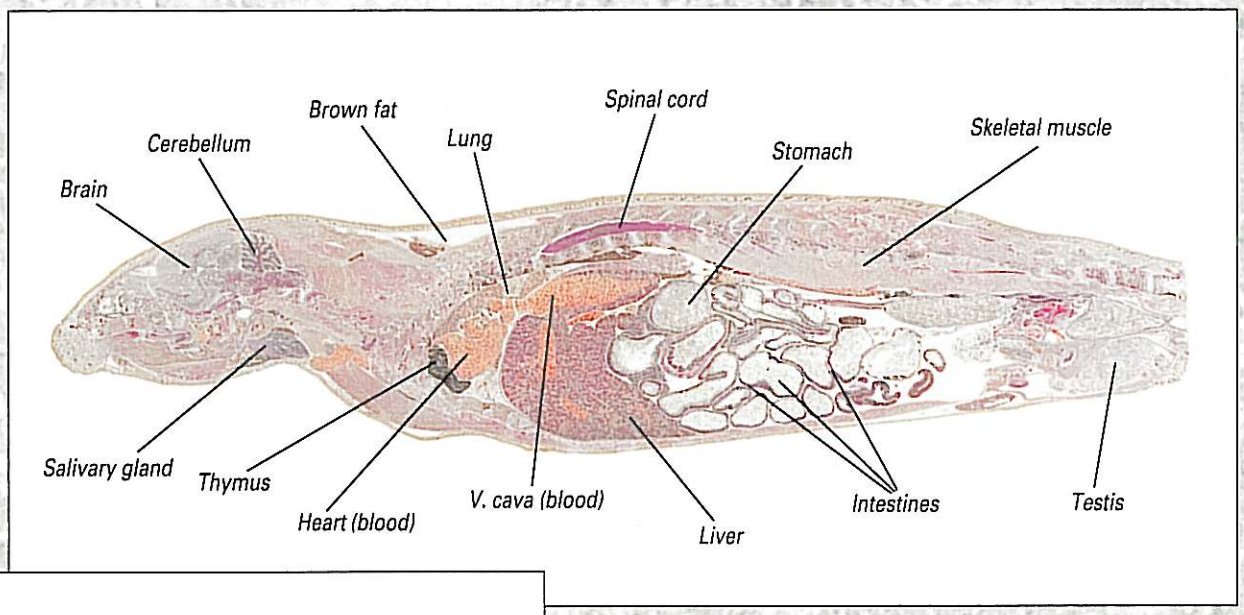
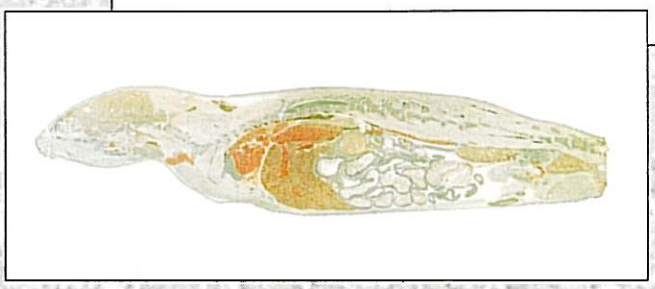
Jung APPLICATIONS BRIEF





The staining of tape-mounted large specimens has gained interest over the past couple of years because of its value in the aid to the identification of various tissues. Staining also provides anatomical and morphological information that is not available from techniques such as whole body autoradiography, and may complement the findings from studies using novel radio-labelled compounds.

This application brief presents a reliable, modified haematoxylin and eosin (HE) staining procedure for tape-mounted rat sections commonly used in pharmaceutical research projects.

A Technique for the Staining of Large Tape-Mounted Sections

Leica

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Sectioning and staining were performed at the Wellcome Research Laboratories in the UK using a Jung Cryomacrocut manufactured by Leica Instruments GmbH, based in Germany. The tape (Tesa-type 4248) used for section retrieval was supplied by the same company.

The clear description of the staining procedure is prompted by the need for a "cookbook-style" report, describing the various steps required in order to succeed in staining tape-mounted cryosections of animal tissues.

Materials and Methods

Microtome: Jung Cryomacrocut heavy-duty cryomicrotome
Sectioning thickness: 15 µm
Sectioning temperature: -20°C
Type of knife used: 35° tool steel knife
Type of tape used: Tesa tape (type 4248), transparent
Specimen: Wistar rat, approximately 200 g
Specimen embedding: Standard commercial carboxymethylcellulose (CMC)

Reagents:

- 10% neutral-buffered formalin was prepared to specification by Pioneer Research Chemicals Ltd., Essex, UK.
- Harris' Haematoxylin (BDH Chemicals Ltd., Poole, Dorset, UK; Prod 35128). Used concentrated.
- Eosin 5% w/v (BDH Chemicals Ltd., Poole, Dorset, UK; Prod 35010). Dilute 1:4 with water.
- Tetenal light-protecting varnish (Tetenal Photowerk GmbH, Norderstadt, Germany; Art No. 2029).

After dosing and killing, the specimen was prepared for whole body autoradiography by deep-freezing the carcass in a mixture of isopentane and solid carbon dioxide, "cardice," at -78°C. The carcass was then embedded in a block of CMC and mounted on the stage of the Cryomacrocut.

At various levels of interest through the specimen, six 15 µm sections were collected onto tape. Five sections were retained for autoradiography and freeze-dried for 24-72 hours at -20°C. The remaining section was air-dried in front of a fan for at least two minutes and stained within two or three days of sectioning.

Staining Procedure

All staining and washing was achieved by laying the tape, section side down, on a reservoir of stain or on the surface of a stream of running water. The tape must not be immersed in the liquids as it becomes easily damaged.

- The section was fixed for two minutes in 10% neutral-buffered formalin and rinsed in running tap water.
- Stain the section in Harris' Haematoxylin for two minutes. Rinse the excess stain from the section and differentiate in 1% acid water (HCI) for 10-30 seconds.
- Blue the section by rinsing in running water for three minutes.
- Counterstain the section in 1% aqueous eosin for 30 seconds. Wash the section well to remove the excess stain.
- Dehydrate the section in glycerol for at least one minute. Blot the section dry and hang until staining is completed.
- The section may either be mounted on acetate or sprayed with a light coat of antifading varnish and mounted in a polythene sleeve.

For more information, please contact the authors or your local sales representative

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