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Histological quantification of thickness of the mucosal and muscle layers of the rat stomach

Madeleine R. Di Natale^{1,2}, Lauren Patten², Martin Stebbing^{1,2}, John Furness^{1,2}¹The University of Melbourne; ²The Florey Institute of Neuroscience and Mental Health

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Madeleine.dinatale

ABSTRACT

The thickness, organization and relationships between the muscle layers of the rat stomach assist in understanding their function. Here we describe protocols for identifying, measuring and quantifying the muscle layers of the rat stomach using histological and microscopy techniques.

We would like to acknowledge Phenomics Australia Histopathology and Slide Scanning Service, University of Melbourne.

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KEYWORDS

Masson's trichrome, rat, stomach, smooth muscle, mucosa, H&E

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GUIDELINES

No specific guidelines for this protocol.

 [RA Lamb Dry Chemical Stains, Haematoxylin \(Weigert A\)](#) **Thermo**

Fisher Catalog #LAMB-250-C Step 8

 [RA Lamb Dry Chemical Stains, Haematoxylin \(Weigert B\)](#) **Thermo**

Fisher Catalog #LAMB-260-C Step 8

 [Histolene](#) **Trajan Scientific and**

Medical Catalog #11031 Step 6

 [Absolute Ethanol](#) **Contributed by users** In 2 steps

 [Mayers hematoxylin solution](#) **Contributed by users** Step 6  [70% Ethanol](#) **Contributed by users** Step 6

 [Sodium bicarbonate](#) **Sigma**

 [Magnesium Sulfate P212121](#) Step 6 **Aldrich Catalog #S6014** Step 6

 [DPX](#) **Merck**

 [Eosin Solution](#) **Contributed by users** Step 6 **Millipore Catalog #1.00579.0500** In 2 steps

 [Fuchsin acid](#) **Bio Basic**

Inc. Catalog #FB0469.SIZE.100g Step 8

 [Ponceau Xylidine](#) **Sigma**

Aldrich Catalog #22308 Step 8

 [Phosphomolybdic Acid solution](#) **Sigma**

Aldrich Catalog #HT153 Step 8

 [Remel™ Light Green Counterstain](#) **Thermo**

Fisher Catalog #R40123 Step 8

 [Acetic acid, glacial](#) **Sigma**

Aldrich Catalog #537020 Step 8

 [Xylene](#) **Bio Basic**

Inc. Catalog #XC9800.SIZE.1L Step 8

SAFETY WARNINGS

Before completing any steps in this protocol please refer to the each SDS/MSDS and follow local safety guidelines set by your institute and manufacturers.

Tissue Collection

- 1 Stomach tissue is collected from adult Sprague-Dawley male and female rats. Rats are supplied with food and water ad libitum prior to any tissue collection. All procedures were approved by The Florey Institute of Neuroscience and Mental Health Animal Ethics Committee.
- 2 Animals are deeply anesthetized with either an intraperitoneal injection of pentobarbital sodium (100mg/kg) or an intraperitoneal injection of a mixture of ketamine (50 mg/kg) and xylazine (10 mg/kg) prior to being perfused transcardially with phosphate buffered saline (PBS: 0.15 M NaCl, 0.01 M sodium phosphate buffer, pH 7.2) followed by fixative.
- 3 Different fixation methods are completed to allow for comparisons, these fixation methods are the following; 4% paraformaldehyde (Sigma Aldrich, USA), 10% neutral buffered formalin (Trajan, Melbourne, Australia) or Zamboni's

fixative (2 % formaldehyde plus 0.2 % picric acid in 0.1 M sodium phosphate buffer, pH 7.0). Some samples are placed into PBS containing nicardipine (1 µm) for 30-40 mins at room temperature before fixation.



Be careful with fixatives. Refer to the each SDS/MSDS and follow local safety guidelines set by your institute and manufacturers.

Histological Staining

- 4 Place tissue into histology cassettes in desired orientation and dehydrate through graded ethanol to histolene before embedding samples in paraffin.
- 5 Cut sections (5 µm) on microtome, dry sections at 37.7 °C and stain with haematoxylin and eosin (H&E). This is completed using a Leica Autostainer XL and Leica CV5030 coverslipper
- 6 Haematoxylin and Eosin staining steps are as follows:

[Histolene Trajan Scientific and](#)

1. Histolene - 5 minutes [Medical Catalog #11031](#)

[Histolene Trajan Scientific and](#)

2. Histolene - 1 minute [Medical Catalog #11031](#)

3. Absolute Alcohol - 1 minute [Absolute Ethanol Contributed by users](#)

4. Absolute Alcohol - 1 minute [Absolute Ethanol Contributed by users](#)

5. 70% Alcohol - 1 minute [70% Ethanol Contributed by users](#)

6. Wash in running tap water

7. Stain in Mayer's Haematoxylin - 6 minutes [Mayers hematoxylin solution Contributed by users](#)

8. Wash in running tap water - 1 minute

9. Differentiate in 0.3% Acid alcohol - 1-2 dips [Acid Alcohol Contributed by users](#)

10. Blue in Scott's tap water (magnesium sulfate buffered with sodium bicarbonate) - 1 minute

[Sodium bicarbonate Sigma](#)

[Magnesium Sulfate P212121](#) [Aldrich Catalog #S6014](#)

11. Wash in running tap water - 3 minute

12. Check microscopically

13. Counter stain with Eosin - 3 minute [Eosin Solution Contributed by users](#)

14. Rinse in running tap water - 1-2 dips

15. 70% Alcohol - 2 dips [70% Ethanol Contributed by users](#)

16. Absolute Alcohol - 4 dips [Absolute Ethanol Contributed by users](#)

17. Absolute Alcohol - 1 minute [Absolute Ethanol Contributed by users](#)

18. Absolute Alcohol - 1 minute [Absolute Ethanol Contributed by users](#)

[Histolene Trajan Scientific and](#)

19. Histolene - 1-2 minutes [Medical Catalog #11031](#)

[Histolene Trajan Scientific and](#)

20. Histolene - 1-2 minutes [Medical Catalog #11031](#)

 [Histolene Trajan Scientific and](#)

21. Histolene - 1-2 minutes **Medical Catalog #11031**

 [DPX Merck](#)

22. Mount in DPX **Millipore Catalog #1.00579.0500**

Results:

Nuclei-blue

Cytoplasm, muscle, collagen, erythrocytes-pink/red

7 Masson's trichrome staining is conducted manually by submerging slides into the solutions described in the steps below.

8 Masson's trichrome staining steps are as follows:

1. Bring sections to water.

2. Place sections in Bouin's fixative - 60 minutes at 60°C

3. Wash in running water - 10 minutes

4. Iron Haematoxylin

4.1 Weigert's A (I part) - 2 minutes

 [RA Lamb Dry Chemical Stains, Haematoxylin \(Weigert A\) Thermo](#)

Fisher Catalog #LAMB-250-C

4.2 Weigert's B (I part) - 2 minutes

 [RA Lamb Dry Chemical Stains, Haematoxylin \(Weigert B\) Thermo](#)

Fisher Catalog #LAMB-260-C

5. Wash in water.

 [Fuchsin acid Bio Basic](#)

6. 1% Ponceau 2 R- (2 parts) + 1 % Acid **Inc. Catalog #FB0469.SIZE.100g**

- (1 Part) -

 [Ponceau Xylidine Sigma](#)

5 minutes **Aldrich Catalog #22308**

7. Wash in water.

 [Phosphomolybdic Acid solution Sigma](#)

8. 1% Phosphomolybdic Acid - 3 minutes **Aldrich Catalog #HT153**

9. Wash in water

 [Remel™ Light Green Counterstain Thermo](#)

10. 1% **Fisher Catalog #R40123**

n - 5 minutes

 [Acetic acid, glacial Sigma](#)

11. Wash and leave in 1% Acetic Acid - 1 minute **Aldrich Catalog #537020**

12. 100% ethanol  [Absolute Ethanol Contributed by users](#)

 [Xylene Bio Basic](#)

13. Dehydrate, clear in xylene **Inc. Catalog #XC9800.SIZE.1L**

 [DPX Merck](#)

14. Coverslip sections using DPX mounting media. **Millipore Catalog #1.00579.0500**

Results:

Nuclei-blue/black

Cytoplasm, muscle and RBC-red

Collagen-blue/green

Histological Quantification

- 9 Examine and image slides using an Axioplan microscope (Zeiss, Sydney, Australia).
- 10 A selection of fiducial points are chosen as sites to measure the thickness of the mucosal and muscle layers in rat stomach.
- 11 At each point three measurements of each muscle layer (mucosa, muscularis mucosae, longitudinal muscle, circular muscle, oblique muscle (where applicable)) are made, the average of these measurements is calculated.
- 12 Average these measurements for all equivalent fiducial points measured.