

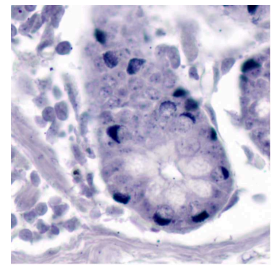
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Version 2

Solcia's lead hematoxylin stain (diffuse endocrinocytes) V.2

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Egor Kuzmin¹

¹Sechenov University



Egor Kuzmin

Sechenov University

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We use this protocol and it's working

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Abstract

Classical histochemical approaches demonstrate considerable diversity in techniques for identifying diffuse endocrine cells, which is directly attributable to the distinct composition of their secretory granules. Among these, the Solcia method stands out as a prominent modification of the MacConaill procedure. Notably, this protocol offers three key advantages: it enables staining without additional section processing, exhibits alcohol resistance (unlike the HCl-toluidine blue method; Solcia, 1968), and critically, functions as a single-step technique – in sharp contrast to multi-step argyrophilic methods (Grimelius 1968) and argentaffin methods (Masson, 1914). Furthermore, the approach facilitates controlled progressive staining through real-time microscopic monitoring, thereby ensuring highly reproducible and morphologically optimal outcomes.

Guidelines

- Reagents must be prepared immediately prior to use. The working solution undergoes rapid oxidation and becomes unsuitable for high-quality staining (maximum stability: 4 hours at room temperature).
- While staining may be performed at elevated temperatures to accelerate reaction kinetics, this markedly increases the risk of precipitate formation. Such particulate matter deposits on specimens, compromising histological integrity. Agitating the solution during staining or repeated immersion/withdrawal of slides is strongly discouraged.
- Post-staining, thorough rinsing in multiple changes of distilled water constitutes a critical step. Due to rapid contaminant accumulation, frequent water replacement is essential.
- Although the original protocol omits acidified water (HCl) and ammoniacal water treatments, empirical evidence indicates their controlled application significantly reduces background staining, eliminates dye-derived precipitates, and enhances chromatic contrast.

Materials

1. Lead nitrate; $\text{Pb}(\text{NO}_3)_2$
2. Ammonium acetate; $\text{NH}_4\text{CH}_3\text{CO}_2$
3. Formalin 10%
4. Hematoxylin
5. Hydrochloric acid; HCL
6. Ortho-xylene; $\text{C}_6\text{H}_4(\text{CH}_3)_2$
7. Ethanol 100% or isopropanol 100%
8. Distilled water

Safety warnings

- !
 - **Formalin** is a volatile compound classified as carcinogenic.
 - **Xylene, isopropyl alcohol (IPA), and ethyl alcohol** are volatile substances hazardous to human health, especially following long-term exposure. Procedures utilizing these solvents require a ventilated fume hood and the use of medical gloves (e.g., nitrile).
 - **Lead** compounds are toxic substances possessing potent oxidizing activity. **Lead(II) nitrate** is recognized as a carcinogen. All procedures involving these compounds must be conducted within a fume hood with appropriate safety precautions to prevent skin and mucous membrane exposure.

Before start

Autopsy specimens were fixed by immersion in neutral buffered formalin for 48 hours. Processing involved a standard protocol utilizing a graded series of isopropyl alcohols, followed by paraffin embedding. Histological blocks were cut on a microtome to yield 3- μm sections. Sections were placed on silane-coated adhesive glass slides for secure adhesion. All staining reagents were freshly prepared immediately before staining commenced.

1 Introduction

Solcia's lead hematoxylin stain

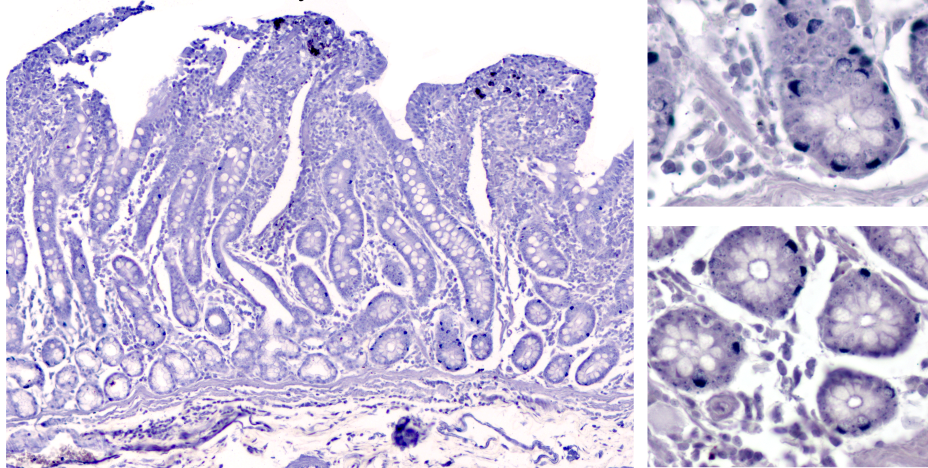


Fig. 1. Micrographs of cow duodenum stained with lead hematoxylin. A positive reaction in the endocrinocytes of the crypt epithelium is shown on the right.

Preparation of solutions

1h

2 Stable lead solution 100 mL

10m

Mix 1:1

1. 40ml **lead nitrate** 5% ($\text{Pb}(\text{NO}_3)_2$ 5g/100ml) solution in distilled water [40 ml]
2. saturated **ammonium acetate** ($\text{NH}_4\text{CH}_3\text{CO}_2$ 60-65%) solution in distilled water [40 ml]



Add

10% formalin (4% formaldehyde) 20 ml per 100 ml of solution [16 ml]

Note

The original protocol uses 40% formaldehyde, which has a concentration 10 times higher, but is less common.

40% formaldehyde 2 ml per 100 ml of solution [1,6 ml]

Stable for weeks at room temperature.

Preparation of solutions

1h



2.1 Working staining solution 100 μ L

40m

Mix 1:1

1. 10 mL **stable lead solution** with
2. 10 mL distilled **water**

Add

0.2 g **hematoxylin** (not hematein).

Stir, let stand 30 minutes, filter.

Dilute to 100 mL

Prepare fresh before use; discard if darkened or precipitated. Stand 30 minutes, filter.

Note

The working solution is unstable—oxidizes within hours. Perform staining at  37–45 °C .
Avoid precipitate artifacts.

2.2 Reducing solution 1 100 mL

5m

HCL-water

2.3 Reducing solution 2 100 mL

5m

Scott's tap water buffer

Protocol

2h 48m 10s

3 Dewaxing, rehydration, rinsing in distilled water.

20m

1. ortho-xylene 5 min.
2. ortho-xylene 5 min.
3. absolute ethanol or isopropanol 5 min
4. 50% ethanol or isopropanol 5 min
5. distilled water 5 min

3.1 Stain in lead hematoxylin working solution for 2 hours at 37 °C

2h

Produce without mixing at a constant temperature



3.2 Rinse in distilled water

5m

Rinse well from sediment



3.3 **Differentiate carefully** (Not obligatory)

Be carried out with caution, staining is very unstable to acidic pH.

10s



3.4 **Rinse in ammonia water for 3 minutes** (Not obligatory)

In order for the hematoxylin to acquire a bluer hue

3m

3.5 **Rinse, dehydrate, mount.**

1. distilled water 5 min
2. 50% ethanol or isopropanol 5 min
3. absolute ethanol or isopropanol 5 min
4. ortho-xylene 5 min.
5. ortho-xylene 5 min.
6. mount

20m

Result

4 Granules of endocrine cells: **dark blue**

(from black to blue depending on the time of exposure in the dye and differentiation)



EC cells of the gastrointestinal tract

AL-cells (X-cell, glucagon) and **G-cells** (gastrin) of the stomach

A-cells (glucagon) and **D-cells** (somatostatin) of the pancreatic islets of Langerhans

Chromophilic cells of the pituitary gland

Cells of the adrenal medulla

Chemoreceptor cells of the carotid body

And other representatives of the diffuse neuroendocrine system

4.1

Expected result

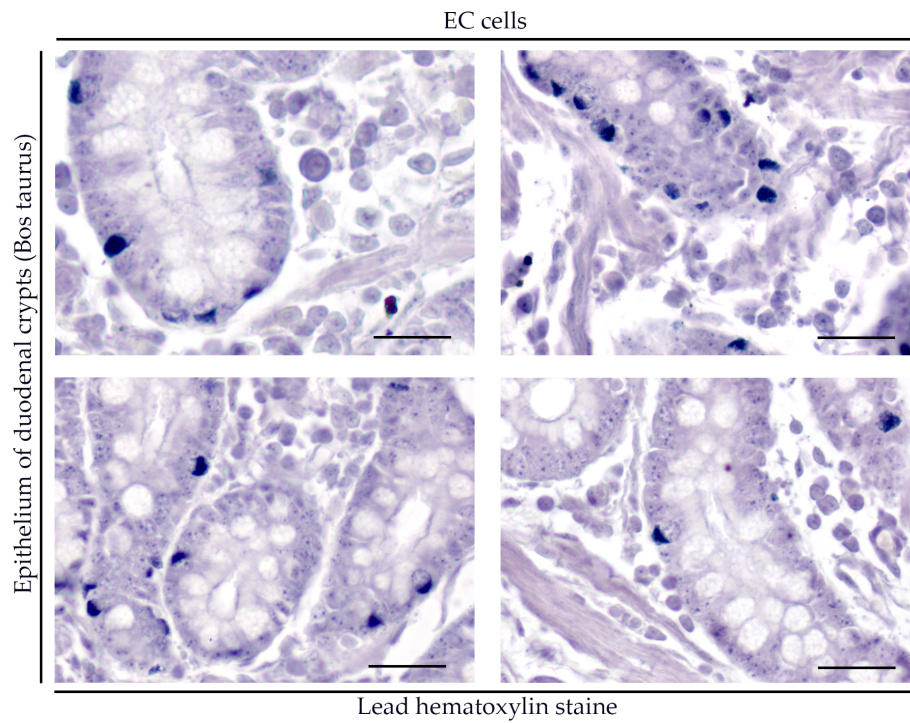


Fig. 2. Microphotographs of bovine duodenum stained with lead hematoxylin. A positive reaction of EC cells is observed at the base of the crypts. Nucleoli are intensely stained, and background nuclear staining is evident.

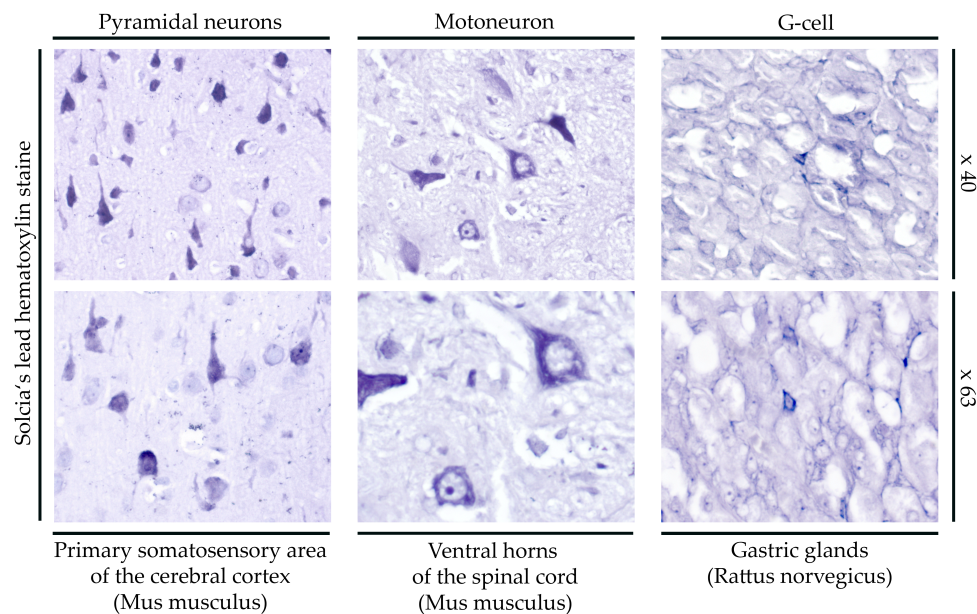


Fig. 3. Microphotographs of sections from various organs stained with lead hematoxylin.

The **right** panel shows a region of Layer V (internal pyramidal lamina) of the primary somatosensory cortex in the mouse brain. The staining highlights the cytoplasm of pyramidal neurons within this layer. Intense staining is observed in specific cortical laminae and in select neurons within subcortical structures. The **center** panel displays the anterior horns of the mouse spinal cord. Intense cytoplasmic staining is evident in the somata of motor neurons, while other neurons remain unstained. The **right** panel features a gastric section from a rat, where scattered endocrine cells (endocrinocytes) are visualized in the mucosa, specifically within the gastric (fundic) glands.

Note

- 5 Pre-oxidation with 1% HCl for 3–17 hours (as in HCl-TB method) improves staining quality.



Protocol references

1. Solcia E, Capella C, Vassallo G. Lead-haematoxylin as a stain for endocrine cells. Significance of staining and comparison with other selective methods. *Histochemie*. 1969;20(2):116-26. **doi: [10.1007/BF00268705](https://doi.org/10.1007/BF00268705)**
2. Solcia E, Vassallo G, Capella C. Selective staining of endocrine cells by basic dyes after acid hydrolysis. *Stain Technol*. 1968 Sep;43(5):257-63. **doi: [10.3109/10520296809115078](https://doi.org/10.3109/10520296809115078)**