



**Diagnostic  
BioSystems**

## Instructions For Use KT 035-IFU

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### Steiner Stain Kit (For Spirochetes)

#### Description and Principle

The Steiner Stain Kit (For Spirochetes) is designed for demonstrating Fungi, Helicobacter Pylori, Legionella pneumophila, and Spirochete infected tissue. Kit may be used on formalin fixed, paraffin-embedded tissue.

Bacteria are sensitized with Zinc Formalin making the organisms receptive to uptake silver from a silver nitrate solution. Silver is then chemically reduced to a visible metallic form with a reducing solution containing hydroquinone. Pretreatment with Oxidizer Solution improves homogenous staining of bacteria and reduces background silver staining.

#### Expected Results

Spirochetes:	Black to Brown
Helicobacter Pylori:	Black to Brown
Fungi:	Black to Brown
Legionella pneumophila	Black to Brown
Background:	Yellow to Tan

#### Kit Contents

Kit Contents	Storage
1. Oxidizer Solution	15-30°C
2. Zinc Formalin Solution	15-30°C
3. Gum Mastic Solution	2-8°C
4. Hydroquinone	15-30°C
5. Silver Nitrate Solution (0.2%)	2-8°C
6. Silver Nitrate Solution (1%)	2-8°C

#### Suggested Controls (not provided)

Helicobacter Pylori infected stomach.

#### Uses/Limitations

For In-Vitro Diagnostic use only.  
Do not use if reagents become cloudy or precipitate  
Do not use past expiration date.  
Use caution when handling reagents.  
Non-Sterile  
Intended for FFPE sections cut at 5-10µm.  
This procedure has not been optimized for frozen sections.  
Frozen sections may require protocol modification.

#### Storage

Mixed storage conditions. Store according to individual label instructions.

#### Safety and Precautions

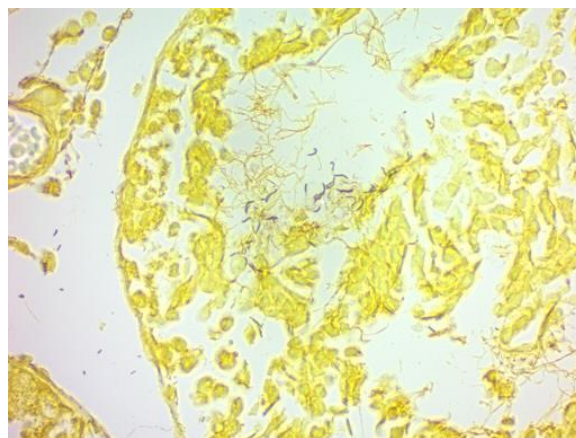
Please see current Safety Data Sheets (SDS) for this product and components GHS classification, pictograms, and full hazard/precautionary statements. If there is any serious incident that has occurred in relation to the device, please contact the manufacturer: Diagnostic BioSystems Technical Support at (925) 484-3350, extension 2 or [techsupport@dbiosys.com](mailto:techsupport@dbiosys.com). If required, please report to the Competent Authority of the Member State in which the user and/or patient is established.

#### Important Notes:

1. All glassware/containers used in this procedure should be chemically cleaned (bleach or acid) and rinsed thoroughly in distilled water.

2. Do not use metal forceps to remove slides from reagents. Use plastic forceps only.

3. Prewarm all reagents to room temperature prior to use.



*Treponema Palidum* spirochetes in Rabbit testes stained with the Steiner Stain Kit (For Spirochetes)

#### Procedure:

##### Prepare Reducing Solution at Step #10 of this Procedure:

Combine:

25ml 1% Hydroquinone (0.25gm Hydroquinone in 25ml Distilled Water)  
15ml Gum Mastic Solution (2.5%)

Mix thoroughly and filter through medium filter paper.

Then Add: 6 Drops (240µl) Silver Nitrate Solution (1%). Mix thoroughly.

1. Preheat Water Bath to 70° Centigrade.
2. Deparaffinize sections if necessary and hydrate to distilled water.
3. Incubate slide in Oxidizer Solution for 20 minutes.
4. Rinse thoroughly in distilled water.

**Note:** Place 20 ml of Silver Nitrate Solution (0.2%) in water bath to preheat.

5. Incubate slide in Zinc Formalin Solution for 5 minutes.
6. Rinse thoroughly in distilled water.
7. Incubate slide in preheated Silver Nitrate Solution (0.2%) for 5 minutes at 70° Centigrade. (Note: Discard solution after this step)
8. Rinse slide thoroughly in distilled water.
9. Dehydrate slide in 2 changes of Absolute Alcohol.

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**Note:** Prepare Reducing Solution (above) and place in water bath to preheat.

10. Incubate slide in Gum Mastic Solution for 3 minutes.
11. Air dry slide for 1 minute or until gum mastic is completely dry.
12. Incubate slide in preheated Reducing Solution for 10-15 minutes or until section is tan to brown at 70° Centigrade. (Note: Discard solution after this step)
13. Rinse slide quickly in distilled water.
14. Dehydrate quickly in 3 changes of absolute alcohol.
15. Clear, and mount in synthetic resin.

### **References**

1. Carson, F.L., (2009), Histotechnology; A Self-Instructional Text, 3rd Edition. ASCP Press, Chicago, IL. Pages 249-250
2. Margeson, L.S., & Chapman, C.M., (1996) Use of Zinc Formalin as a Sensitizer in Silver Stains for Spirochetes, Journal of Histotechnology, 19:2, 135-138, DOI: 10.1179/his.1996.19.2.135
3. Swisher, B.L., (1987) Modified Steiner Procedure for Microwave Staining of Spirochetes and Nonfilamentous Bacteria, Journal of Histotechnology, 10:4, 241-243, DOI: 10.1179/his.1987.10.4.241



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