

Warthin-Starry Stain Kit

Catalog No.	BP2917 100 Test
Introduction	This Warthin-Starry Stain Kit demonstrates <i>Helicobacter pylori</i> , <i>Legionella pneumophila</i> , and Cat Scratch Fever bacteria. It is suitable for any well fixed paraffin embedded tissue cultures cut at 4 microns.
Format	Kit consists of 0.5% Silver Nitrate, Acidulated (125 ml), 4% Gelatin, Acidulated (125 ml), 2% Silver Nitrate, Acidulated (30 ml), 0.1% Hydroquinone, Acidulated (2 x 30 ml) and Spirochete Sensitizer (125 ml).
Use	For <i>Helicobacter pylori</i> , <i>Legionella pneumophila</i> , and Cat Scratch Fever bacteria staining.
Storage	Store all solutions at 2-8°C except the Spirochete Sensitizer, which is stored at room temperature.
Expiry	Do not use beyond expiration date indicated on the product label.

Protocol (Modified Stat)

Reducing Solution

Prepared at Step #5

1. Warm 4% Gelatin, Acidulated to liquefy.

4% Gelatin, Acidulated	12.5 ml
2% Silver Nitrate, Acidulated	20 to 30 drops
0.1% Hydroquinone, Acidulated	7.5 ml
2. Prepare reducing solution in an unused plastic Five Slide Mailer or Slide Jar.
3. Mix components thoroughly or uneven staining will result.

Procedure

1. Deparaffinize slide using Xylene or a Xylene Substitute and hydrate through alcohols to Distilled water.
2. For spirochete staining only, place slide in room temperature Spirochete Sensitizer for 5 minutes, then rinse slide thoroughly in distilled water.

NOTE: For all other listed bacteria, Step 2 may be omitted. If Spirochete Sensitizer is not used, it will not affect correct demonstration of *H. pylori*, *L. pneumophila*, or Cat Scratch Fever bacteria.

3. Put 20 ml of 0.5% Silver Nitrate, Acidulated in an unused plastic Slide Jar.
4. Place the Slide Jar of 0.5% Silver Nitrate, Acidulated in a waterbath at 65° to 70°C for 5 minutes.
5. Place slide into the hot 0.5% Silver Nitrate, Acidulated, agitate slide thoroughly and incubate for 3 to 5 minutes.
6. Transfer slide from the 0.5% Silver Nitrate, Acidulated into the Reducing Solution. Place container into a 65° to 70°C waterbath, agitating frequently (This step may take 1 to 5 minutes) until the section is brown.

NOTE: If slide is not agitated frequently during this step, uneven staining can occur.

7. Rinse slide carefully in hot Tap water for 2 minutes.
8. Dehydrate slide through 3 changes of fresh Absolute Alcohol.
9. Clear slide through 3 changes of fresh Xylene or Xylene Substitute.
10. Coverslip slide immediately using a permanent mounting media.

Results

Helicobacter pylori, *Legionella pneumophila*:
Spirochetes, Cat Scratch Fever bacteria:
Background:

Dark Brown, Gray or Black
Dark Brown, Gray or Black
Yellow to Brown

Use of the Warthin-Starry Stain Kit does not guarantee the successful outcome of any histological staining analysis.
Optimal dilution and reaction conditions to be determined by investigator usage.

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Storage	Store all solutions at 2-8°C except the Spirochete Sensitizer, which is stored at room temperature.
Expiry	Do not use beyond expiration date indicated on the product label.

Protocol (Microwave Oven)

Reducing Solution

Prepared at Step #7

1. Warm 4% Gelatin, Acidulated to liquefy.
4% Gelatin, Acidulated 12.5 ml
2% Silver Nitrate, Acidulated 20 to 30 drops
0.1% Hydroquinone, Acidulated 7.5 ml
2. Prepare reducing solution in an unused plastic Five Slide Mailer or Slide Jar.
3. Mix components thoroughly or uneven staining will result.

Procedure

A 500 watt microwave oven was used to develop this procedure.

1. Deparaffinize slide using Xylene or a Xylene Substitute and hydrate through alcohols to Distilled water.
2. For spirochete staining only, place slide in room temperature Spirochete Sensitizer for 5 minutes, then rinse slide thoroughly in distilled water.

NOTE: For all other listed bacteria, Step 2 may be omitted. If Spirochete Sensitizer is not used, it will not affect correct demonstration of *H. pylori*, *L. pneumophila*, or Cat Scratch Fever bacteria.

3. Put 20 ml of 0.5% Silver Nitrate, Acidulated in an unused plastic Slide Jar.
4. Put 20 ml of Tap water into a Coplin jar, then place slide jar into the Coplin jar.
5. Place the Coplin Jar in a microwave oven and heat for 30 seconds at full power.
6. Place slide into the 0.5% Silver Nitrate, Acidulated. Cap Slide Jar tightly and invert several times to equalize solution temperature, then return Slide Jar to Coplin jar and incubate for 5 minutes.
7. Remove Slide Jar from Coplin jar and replace with Slide Jar containing the Reducing Solution and heat for 20 seconds at full power. Remove Coplin jar from microwave and transfer slide into the Reducing Solution. Agitate slide thoroughly and incubate for 2 to 5 minutes; remove slide when section is brown.
8. Rinse slide carefully in hot Tap water for 2 minutes.
9. Dehydrate slide through 3 changes of fresh Absolute Alcohol.
10. Clear slide through 3 changes of fresh Xylene or Xylene Substitute.
11. Coverslip slide immediately using a permanent mounting media.

Results

<i>Helicobacter pylori</i> , <i>Legionella pneumophila</i> :	Dark Brown, Gray or Black
Spirochetes, Cat Scratch Fever bacteria:	Dark Brown, Gray or Black
Background:	Yellow to Brown

Use of the Warthin-Starry Stain Kit does not guarantee the successful outcome of any histological staining analysis.
Optimal dilution and reaction conditions to be determined by investigator usage.