# ab287884 – GMS Staining ELISA Kit

For use in the histologic visualization of fungi, basement membrane and some opportunistic organisms such as Pneumocystis carinii

For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit:

http://www.abcam.com/ab287884

## Storage and Stability

All the reagents are shipped at room temperature (RT) and stored at multiple temperatures.

## **Materials Supplied**

Item	Quantity	Storage Condition
Silver Nitrate Solution (0.2%)	125 mL	2-8°C
Methenamine Solution	125 mL	2-8°C
Gold Chloride Solution (0.2%)	125 mL	2-8°C
Borax Solution	15 mL	RT
Sodium Bisulfite Solution	125 mL	RT
Chromic Acid Solution	125 mL	RT
Sodium Thiosulfate Solution (5%)	125 mL	RT
Light Green Solution	125 mL	RT

# Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully utilize this assay:

- Distilled water
- Coplin jars
- Plastic forceps
- Absolute alcohol
- Synthetic resin

#### **Reagent Preparation**

- Do not use if reagents become cloudy.
- Do not use past expiration date.
- Use caution when handling reagents.
- All glassware used in this procedure should be chemically cleaned and rinsed thoroughly in distilled water.
- Failure to adequately remove the alcohol used in departifination will result in reduction of the chromic acid solution.
- Reduction of the chromic acid solution will result in a change in color from orange to brown. Discard the reagent if color change is noted.
- Do not use metal forceps to remove slides from reagents. Use plastic forceps only.
- Pre-warm all reagents to room temperature prior to use.

#### **Assay Procedure - Standard**

- 1. Deparaffinize sections if necessary and hydrate to distilled water.
- 2. Incubate slide in Chromic Acid Solution for 10 minutes.
- 3. Rinse in tap water followed by 2 changes of distilled water.
- Incubate slide in Sodium Bisulfite Solution for 1 minute (to remove any residual chromic acid).

- 5. Rinse in tap water followed by 2 changes of distilled water.
- 6. Combine the following for a working GMS solution:
  - a. 25 ml Silver Nitrate Solution (0.2%)
  - **b.** 25 ml Methenamine Solution
  - c. 2 ml Borax Solution

 $\Delta$ **Note:** Mixed solution may not be stored for reuse later.

- 7. Place working GMS solution in 60° centigrade water bath and allow temperature to equilibrate.
- 8. Incubate slide in working GMS solution for 10-15 minutes. Using plastic forceps, dip slide in distilled water and check under a microscope for evaluation of silver impregnation. Fungi should be dark brown. If color is not sufficient, return the slide to working GMS solution for 2-3 minutes and check again.
- 9. Rinse in 4 changes of distilled water.
- 10. Incubate slide in Gold Chloride Solution for 15-30 seconds.
- 11. Rinse in 4 changes of distilled water.
- 12. Incubate slide in Sodium Thiosulfate Solution (5%) for 2 minutes.
- 13. Rinse in tap water followed by 2 changes of distilled water.
- 14. Incubate slide in Light Green Solution for 2 minutes.
- 15. Rinse slide using absolute alcohol.
- 16. Dehydrate in 2 changes of absolute alcohol, clear, and mount in synthetic resin.

#### Assay Procedure - Microwave

**Note:** These instructions were developed using a standard 500-watt microwave oven. Heating times should be modified as needed depending on the microwave oven used.

- 1. Deparaffinize sections if necessary and hydrate to distilled water.
- 2. Place slide in plastic coplin jar filled with Chromic Acid solution. Cap jar loosely!
- 3. Place jar in microwave oven and heat on high power for 10 seconds. Allow slide to remain in warm solution for 3 minutes.
- 4. Rinse in tap water followed by 2 changes of distilled water.
- 5. Incubate slide in Sodium Bisulfite solution for 1 minute (to remove any residual chromic acid).
- 6. Rinse in tap water followed by 2 changes of distilled water.
- 7. Combine the following for a working GMS solution:
  - a. 25 ml Silver Nitrate
  - **b.** 25 ml Methenamine
  - c. 2 ml Borax Solution

 $\Delta$ **Note:** Mixed solution may not be stored for reuse later.

- 8. Place working GMS solution (loosely capped) in microwave oven for 40 seconds. Remove and pour several times between coplin jar and a clear graduated cylinder to mix thoroughly (use protective glove to avoid burning hand). Mixed solution remains in coplin jar.
- 9. Incubate slide in working GMS solution (heated) for 2-6 minutes until the tissue is medium brown in color. Using plastic forceps, dip slide in distilled water and check under a microscope for evaluation of silver impregnation. Fungi should be dark brown. If color is not sufficient, return he slide to working GMS solution for 1-2 minutes and check again. Reheat solution if needed.
- 10. Rinse in 4 changes of distilled water.

- 11. Incubate slide in Gold Chloride solution for 15-30 seconds.
- 12. Rinse in 4 changes of distilled water.
- 13. Incubate slide in Sodium Thiosulfate for 2 minutes.
- 14. Rinse in tap water followed by 2 changes of distilled water.
- 15. Incubate slide in Light Green Solution for 2 minutes.
- 16. Rinse slide using absolute alcohol.
- 17. Dehydrate in 2 changes of absolute alcohol, clear, and mount in synthetic resin.

# **Technical Support**

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