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## Staining Spores for Conidia Counting

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**Protocol status:** Working

**We use this protocol and it's working**

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### Abstract

Methylene blue has different functions depending on its concentration. Its use allows the visualization of dead tissues and bacteria, for example. As a solution, this dye allows the identification of microorganisms in general. For practical use in this matter, the morphological identification of the microorganism is obtained from the visualization of its conidia with the specific dye.



## Procedure

- 1 Add sample in 1,5 mL tube;
- 2 Add 100 uL of methylene blue dye;
- 3 Homogenize by vortexing for 10 seconds and wait 2 minutes;
- 4 Centrifuge for 1 minute at 16.000g;
- 5 Pipette 50 uL into the Neubauer chamber or microscope slide and cover with a cover slip;
- 6 Evaluate under an optical microscope;
- 7 Store at 4°C overnight before to use.

## Materials

- 8 Tween® 80;
- 9 Methylene blue;
- 10 Distilled water.

## Solution Preparation (100 mL)

- 11 In a becker add 80 mL of distilled water;



- 12 Weigh 0.7g of methylene blue;
- 13 Pipette out 20 uL of Tween® 80;
- 14 Fill volume to 100 mL with distilled water.

## References

- 15 Pham, Tuan Anh et al. "Production of Blastospore of Entomopathogenic *Beauveria bassiana* in a Submerged Batch Culture." *Mycobiology* vol. 37,3 (2009): 218-24.  
doi:10.4489/MYCO.2009.37.3.218
- 16 Oktari, A., et al. "The bacterial endospore stain on Schaeffer Fulton using variation of methylene blue solution." *Journal of Physics: Conference Series*. Vol. 812. No. 1. IOP Publishing, 2017.