

ALKALINE METHYLENE BLUE SOLUTION

Methylene Blue Solution

Citric Acid

0.1M Glycine Buffer (pH 10.6)

2 small, clean (100-125ml) Glass Bottles

Sterile Pipets

Distilled Water

Mechanical Pipeter

Foil

Procedure:

1. Weigh 0.1g Methylene Blue and place in small glass bottle.
2. Add distilled water up to 100ml mark on bottle.
3. Swirl bottle to dissolve media. (This is your 0.1% Methylene Blue stock solution.)
4. Place 10ml of the stock solution into a new glass bottle.
5. Dilute stock Methylene Blue solution up to 100ml mark with the 0.1M glycine buffer. This solution is ready to use for vitality tests.
6. Place 500 μ l of the “ready to use” methylene blue solution into 1.5ml eppendorf tubes for cell & vitality counting.

Note: Discard solution after 6 months.