



Focal Points



Application Note FP-149

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Multi-Color Bacterial Counting With the ColonyDoc-It™ Imaging Station

Introduction

Chromogenic agents assist researchers in identifying bacteria of specific metabolism by color. The chromogenic substrate X-Gluc is used in a variety of applications for the detection of the β -glucuronidase enzyme. Upon reduction, X-Gluc produces a localized color, making it useful in identifying *GUS* gene presence. X-Gluc has reported applications in the detection of contaminated food samples such as meat, dairy, and shellfish products. The ColonyDoc-It Imaging Station (Figure 1) has the ability to differentiate between β -glucuronidase positive (blue) bacteria and negative (yellow) bacteria. Figure 2 shows a sample of multicolor colonies captured with the ColonyDoc-It Station.



Fig. 1 – ColonyDoc-It Imaging Station

Materials

- LB agar with 0.2g/L X-Gluc (catalogue # G1281C1, Gold BioTechnology, Inc., St. Louis, MO)
- Environmental sample in water

Sample Preparation

- Spread the LB agar plate evenly with the diluted environmental sample. Use a series of dilution plates if the bacteria are too concentrated and touching.
- Incubate the agar plates overnight at 37°C.
- Store the plate at 4°C. Image after 48 hours.

Note: For best results, 20 – 200 bacterial colonies should be counted.

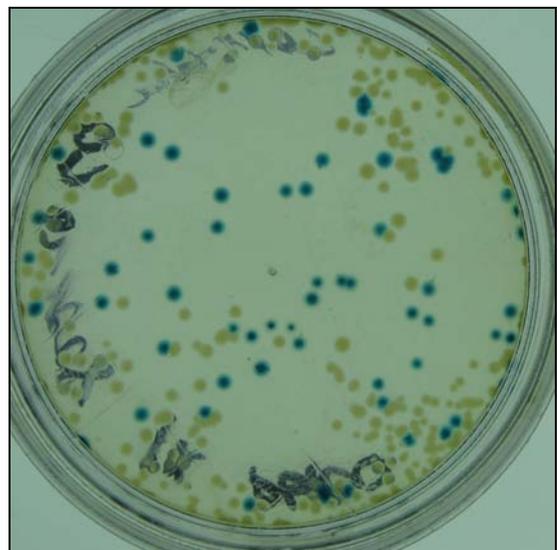


Fig. 2 – Multicolor Colonies

Imaging Methods

- In the software, click onto the appropriate plate size, select the smallest colony option and chose White Light for lighting.
- To capture an image of the plate, select Capture in the software. An image resembling the one in Fig. 3 will appear.
- Click the **Start Colony Count** Button then choose the Manual Counting process.



Fig. 3 – ColonyDoc-It Software Interface

- Define the region of interest (Counting Region) or use the default ROI that outlines the image of the plate in green.
- Set up the classes by clicking on the blue colonies. Adjust the point sensitivity to highlight all the blue colonies in white. The image with the blue colonies identified will show black-and-white in the Colony Count Wizard window – Figure. 4.
- Add another class by clicking on one of the yellow colonies. Adjust the point sensitivity in the same way as the blue colonies.
- Click Count to go to the last step in the colony count wizard. If necessary, adjust the shape and size slide bars to capture more colonies in the final step.

Note: A Template can be set up at this step to minimize the counting steps for similar plates in the future.

- Click **Finish** to complete the counting process.

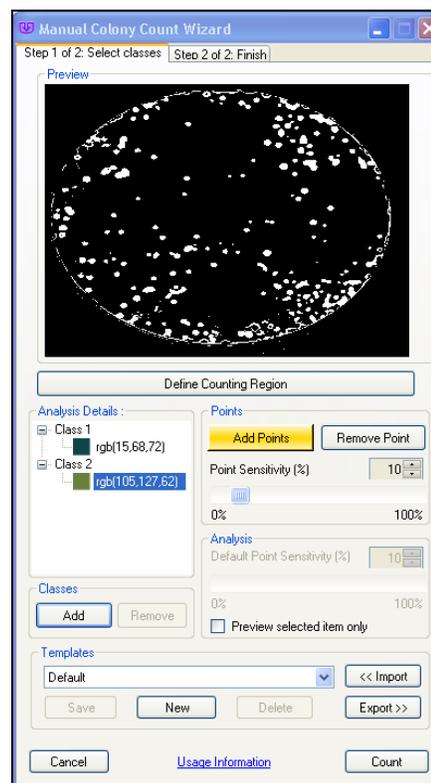


Fig. 4 –Colony Count Wizard

Results

The results window shows that a total of 225 colonies were counted with 54 class 1 (blue) colonies and 171 class 2 (yellow) colonies. Approximately 24% of the population was found positive in β -glucuronidase.

