



# Focal Points



## Application Note FP-148

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## Counting GFP Inhibition Zones with the ColonyDoc-It™ Imaging Station

### Introduction

Researchers employ the characteristics of GFP (Green Fluorescent Protein) in inhibition zone measurements to clearly identify areas of sensitivity to bacteria. Disks soaked in antibiotic are placed in a Petri dish growing bacteria that expresses GFP. If the bacteria are susceptible to the antibiotic, the bacteria's growth will be inhibited around the disk soaked in antibiotic. The susceptible area will show a clearing of GFP bacteria around the disk. The size of the area cleared of GFP bacteria can be measured to determine total susceptibility.

The ColonyDoc-It Imaging Station (Fig. 1) can be used for effective inhibition zone sizing.



Fig. 1 – ColonyDoc-It Imaging Station

### Materials

- Transformed E-Coli expressing GFP
- 10mL LB medium
- 10cm LB agar plates
- Antibiotic susceptibility discs
- Absorption spectrometer

### Methods

#### Preparation

- Suspend a colony of the bacteria of interest in the suitable medium and incubate until its absorption reaches 0.5OD at 600nm.
- Spread 1mL of the bacteria in suspension on a 10cm diameter plate. (Dispense 2.25 mL if working with a 15cm diameter plate.)

**Note:** It is very important that the dispensed liquid fully covers the surface of the agar.

- Use a sterile tweezer to place the paper disc loaded with antibiotics on the surface of the agar. Ensure that the spacing between disks placed on the agar is even.
- Culture at 37°C overnight.

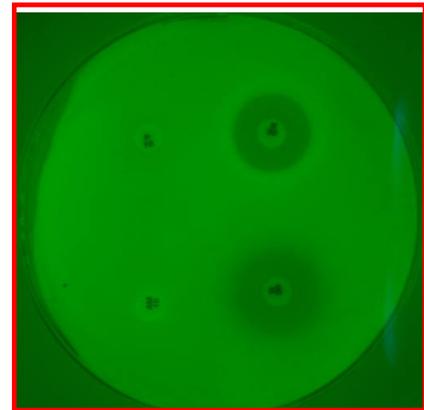


Fig. 2 - Plate demonstrating zone of inhibition

## Capturing the Image

- Place the black plate inside the ColonyDoc-It station. Place the Petri dish with the GFP expressing E-Coli and disks on top of the black plate. The Petri dish can be upside-down if the medium is transparent.
- Turn on the epi-blue lighting switch on the front of the ColonyDoc-It unit. (Ensure all other sources of light are turned off.)
- From the ColonyDoc-It software (Fig. 3) camera control panel, select the **Plate Diameter** size for the inhibition zone sample.
- Select the **Smallest Colony** size in the plate.
- Close the ColonyDoc-It doors to block the ambient light.
- Select **Preview** to ensure that the plate fits inside the region of interest.
- Select **Fluorescent** colony lighting and move the Dim/Bright slider to adjust the appearance of the previewed image.
- Ensure that the GFP filter is positioned just below the camera so that the image appears green. Move the filter if necessary.
- Once positioned correctly, click the **Capture** button to take a picture of the colonies.

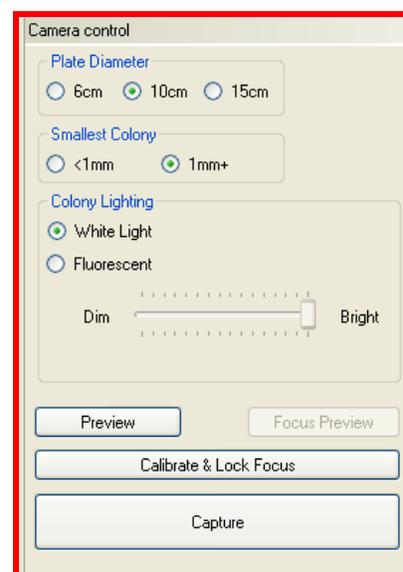


Fig. 3 - Camera Control Software Interface

## Generate Inhibition Zone Sizing

- To measure the size of the zones, open the Colony Counting plugin and select **Start Colony Count**. Click on the checkbox for **zone sizing** and then select **Manual count**. Click on a point within the zone (the area around the antibiotic susceptibility disk that is clear of bacteria). The software will automatically match all similar points in other zones. Adjust the sensitivity of the point selected until the entire zone area is highlighted. Click **Count**.
- Note: If performing inhibition zone sizing on similarly cultured (medium and bacteria) plates, save the parameters used in the manual count window as a template to speed the process for subsequent plates.

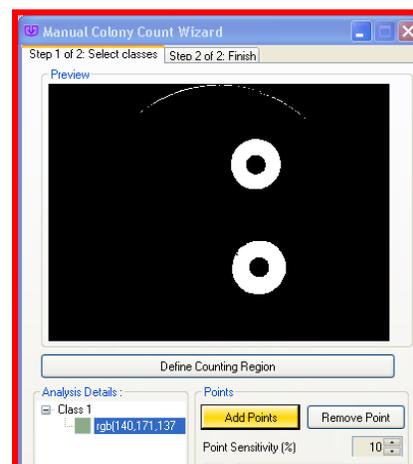


Fig. 4 - Manual Count Software Interface

## Results

The software found two inhibition zones and created a detailed report of each zone counted. Results are automatically entered into a tabular format and can be exported into Excel. The reported parameters are: classes, colonies, statistics, and distribution of colony area.

- The classes parameter of the report details what number and type of zones were detected in the count.
- The colonies parameter lists each zone by number and provides information related to the class, area, perimeter, average diameter, and circularity of each zone counted.
- The statistics parameter of the report presents the numerical value of each value in a statistical format.
- The distribution parameter provides a bar graph of each zone area.

Colony Number	Class	Area(px)	Perimeter(px)	Avg Diameter(px)	Circularity
1	1	6865	311.25	63.63	1.12
2	1	8012	346.67	101.24	1.2

Fig. 5 - Results

## Conclusion

The ColonyDoc-It Imaging Station provides an easy method to capture, count, and report sizing information for inhibition zones. By providing researchers with a Colony Counting software module, zone sizing in antibiotic testing is achieved simply and accurately.

ColonyDoc-It is a registered trademark of UVP, LLC.