G-Biosciences ♦ 1-800-628-7730 ♦ 1-314-991-6034 ♦ technical@GBiosciences.com

A Geno Technology, Inc. (USA) brand name

Antibiotic Sensitivity & Bacteria Screening

Teacher's Guidebook

(Cat. # BE-203)



MATERIALS INCLUDED 3
SPECIAL HANDLING INSTRUCTIONS 3
ADDITIONAL EQUIPMENT REQUIRED 3
TIME REQUIRED 3
OBJECTIVES 4
BACKGROUND 4
TEACHER'S PRE EXPERIMENT SET UP 5
MATERIALS FOR EACH GROUP 5
PROCEDURE 6
RESULTS, ANALYSIS & ASSESSMENT 7

MATERIALS INCLUDED

This kit has enough materials and reagents for 24 students (six groups of four students).

- 10 Discs: Ampicillin Discs
- 10 Discs: Penicillin Discs
- 10 Discs: Tetracycline Discs
- 10 Discs: Nalidixate Discs
- 10 Discs: Blank Discs
- 1 vial E.C. K-12 Agar Stab
- 1 vial LB Broth
- 1 bottle PBS
- 1 pack LB Agar
- 6 Petri Dishes
- 6 Forceps

SPECIAL HANDLING INSTRUCTIONS

- Store E.C. K-12 Agar Stab and all antibiotic discs at 4°C.
- All other reagents can be stored at room temperature.
- Briefly centrifuge all small vials before opening to prevent waste of reagents.

ADDITIONAL EQUIPMENT REQUIRED

- Shaking Incubator
- Incubator
- Autoclave*

** 100ml premade bottles of LB agar (L011), which is melted in a boiling waterbath, can be used if an autoclave is not available. You will require 2 x LB Agar bottles.*

TIME REQUIRED

- Day 1: 30minutes
- Day 2: 30minutes

OBJECTIVES

- Perform antibiotic screening procedure.
- Observe the effects of antibiotics on bacteria.

BACKGROUND

This kit teaches manipulation and use of bacteria in various screening techniques. Specifically, this kit teaches the basic principles of antibiotics, bacterial resistance and susceptibility. Students learn and understand the use of antibiotic resistance in screening for infectious diseases. Utilizing a bacterial strain, students learn the effects of different antibiotics and visualize bacterial sensitivity and resistance to several antibiotics. This involves the use of filter paper discs impregnated with specified concentrations of antibiotics on the surface of agar plate containing bacteria.

This kit will enable students to analyze the inhibitory effects of different antibiotics on selected bacterial cells, based on which they will learn how to decide on the correct course of treatment for a particular bacterial infection.

TEACHER'S PRE EXPERIMENT SET UP



Wear heat protective gloves throughout the autoclaving and pouring agar plate procedure.

Make Agar plates the day before the experiment. Agar plates can be made up to a week in advance, stored in an airtight container at 4 ℃.

1. Add the entire pack of LB Agar to an autoclavable container and add 150ml distilled water. Autoclave for 15min at 121°C.

2. Once the LB Agar has cooled to handle (about 45°C), pour a ~0.5cm / ¼" layer of agar into six Petri dishes. This is approximately 20-25ml each plate.

3. Let the plates set for 20-30 minutes to solidify. Store upside down in an airtight container until required.

4. Transfer 0.8ml LB broth to the bacterial agar stab and incubate at 37°C for 30 minutes. Retain the vial of LB Broth.

5. Vigorously shake or vortex for 1-2 minutes, then transfer 0.5ml LB broth from the agarose stab to the LB Broth vial. Incubate at 37°C overnight in a shaking incubator.

6. The next day, pipette 1ml overnight culture to the bottle of PBS. Mix thoroughly by swirling and dispense 2ml PBS culture suspension in to sterile tubes for each student group.

   Note: *Teacher can choose different testing bacteria instead of the E.C. K-12 supplied with this kit.*

MATERIALS FOR EACH GROUP

Supply each group with the following components.

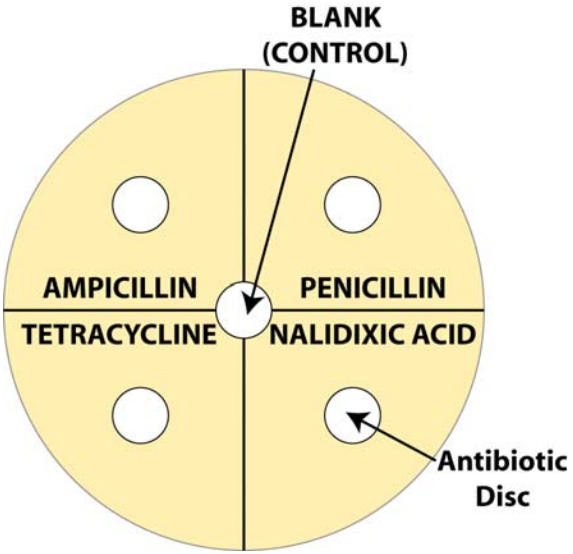
- 1 Ampicillin Disc
- 1 Penicillin Disc
- 1 Tetracycline Disc
- 1 Nalidixate Disc
- 1 Blank Disc
- 1ml E.C. K-12 suspension
- 1 LB Agar Plate
- 1 Forceps
- Marker pen

PROCEDURE



Wear gloves throughout the experiment procedure.

1. Divided the bottom of the LB Agar Plate into 4 areas and label the areas according to the antibiotic to be tested: Ampicillin, Penicillin, Tetracycline and Nalidixic acid. Mark the center as the blank control (Figure 1). DO NOT APPLY THE DISCS.



2. Pipette 1ml E.C. K-12 suspension on to the plate and gentle swirl and rock the plate to ensure the bacterial suspension covers the entire surface of the plate.

3. Remove the excess bacterial suspension and let the plate dry for 10-15minutes.

4. Using forceps, carefully place the blank disc in the center of the plate. Place the 4 antibiotic discs in the center of the appropriately marked area.

5. Replace lid, invert the plate and incubate in a 37°C incubator for 12 to 24 hours.

6. Observe the plate and measure the diameter of the inhibition rings.

RESULTS, ANALYSIS & ASSESSMENT

Measure the size of the clear areas around the antibiotic discs. These areas represent inhibition of bacterial growth for a given concentration of antibiotic.

Diameter of Ampicillin inhibition ring: _____

Diameter of Penicillin inhibition ring: _____

Diameter of Tetracycline inhibition ring: _____

Diameter of Nalidixate inhibition ring: _____

Is there a difference in the inhibitory effect of the various antibiotics, if so why?

The size of the inhibition ring depends on many factors. The sensitivities of bacteria to different antibiotics are different. The concentration of antibiotic, the density of bacteria on the plate and the thickness of the agar plate all affect the inhibition ring size.

Last saved: 9/5/2012 CMH





www.GBiosciences.com

G-Biosciences ♦ 1-800-628-7730 ♦ 1-314-991-6034 ♦ technical@GBiosciences.com

A Geno Technology, Inc. (USA) brand name

Antibiotic Sensitivity & Bacteria Screening

Student's Handbook

(Cat. # BE-203)



OBJECTIVES 3
BACKGROUND 3
MATERIALS FOR EACH GROUP 3
PROCEDURE 4
RESULTS, ANALYSIS & ASSESSMENT 5

OBJECTIVES

- Perform antibiotic screening procedure.
- Observe the effects of antibiotics on bacteria.

BACKGROUND

This kit teaches manipulation and use of bacteria in various screening techniques. Specifically, this kit teaches the basic principles of antibiotics, bacterial resistance and susceptibility. Students learn and understand the use of antibiotic resistance in screening for infectious diseases. Utilizing a bacterial strain, students learn the effects of different antibiotics and visualize bacterial sensitivity and resistance to several antibiotics. This involves the use of filter paper discs impregnated with specified concentrations of antibiotics on the surface of agar plate containing bacteria.

This kit will enable students to analyze the inhibitory effects of different antibiotics on selected bacterial cells, based on which they will learn how to decide on the correct course of treatment for a particular bacterial infection.

MATERIALS FOR EACH GROUP

Supply each group with the following components.

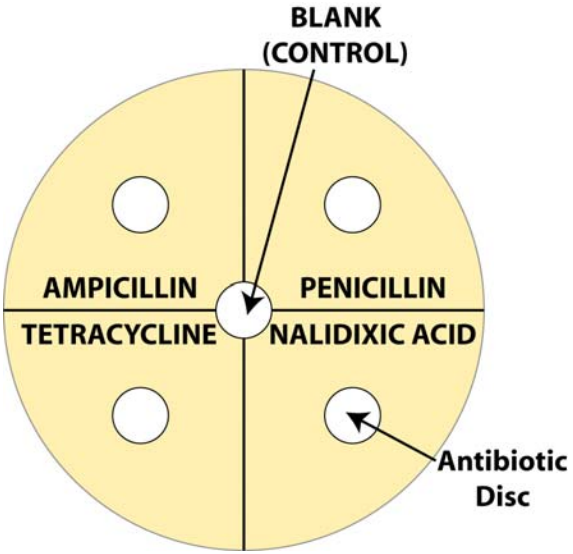
- 1 Ampicillin Disc
- 1 Penicillin Disc
- 1 Tetracycline Disc
- 1 Nalidixate Disc
- 1 Blank Disc
- 1ml E.C. K-12 suspension
- 1 LB Agar Plate
- 1 Forceps
- Marker pen

PROCEDURE



Wear gloves throughout the experiment procedure.

1. Divided the bottom of the LB Agar Plate into 4 areas and label the areas according to the antibiotic to be tested: Ampicillin, Penicillin, Tetracycline and Nalidixic acid. Mark the center as the blank control (Figure 1). DO NOT APPLY THE DISCS.



2. Pipette 1ml E.C. K-12 suspension on to the plate and gentle swirl and rock the plate to ensure the bacterial suspension covers the entire surface of the plate.

3. Remove the excess bacterial suspension and let the plate dry for 10-15minutes.

4. Using forceps, carefully place the blank disc in the center of the plate. Place the 4 antibiotic discs in the center of the appropriately marked area.

5. Replace lid, invert the plate and incubate in a 37°C incubator for 12 to 24 hours.

6. Observe the plate and measure the diameter of the inhibition rings.

RESULTS, ANALYSIS & ASSESSMENT

Measure the size of the clear areas around the antibiotic discs. These areas represent inhibition of bacterial growth for a given concentration of antibiotic.

Diameter of Ampicillin inhibition ring: _____

Diameter of Penicillin inhibition ring: _____

Diameter of Tetracycline inhibition ring: _____

Diameter of Nalidixate inhibition ring: _____

Is there a difference in the inhibitory effect of the various antibiotics, if so why?

_____

_____

_____

_____

Last saved: 9/5/2012 CMH

This page is intentionally left blank

This page is intentionally left blank





www.GBiosciences.com