



# Gram Staining

**STEM Sims**

## Lesson 1: Practicing the Gram Stain with a Broth Culture

Gram staining is a process used to help identify bacteria based on how they react to a series of reagents. In this lesson, you'll practice applying these reagents to a broth culture of bacteria, and then identify the results of your test.

### Doing the Science

1. Start the Gram Staining Simulation by clicking on the "Sim" tab.
2. Click the "Practice" button.
3. Click on the marker and select the "Broth 18 hours" option.
4. Click on the inoculation loop and click "sterilize."
5. Again, click on the inoculation loop and select the "Broth 18 hours" option.
6. After the inoculation loop has retrieved some culture from the plate, click the inoculation loop again and select a dime-sized smear.
7. Sterilize the inoculation loop again.
8. Click on the slide and select air-dry for 5 minutes.
9. Click on the slide and select a heat fix with 5 passes through the flame.
10. Click the "Done" button to store the smear.
11. Click on the "Gram Staining" button.
12. Click the "Select Slide" button and select the slide you prepared.
13. Flood the smear with Crystal Violet at point A with a flat orientation for 60 seconds by clicking the "Add" button, then selecting Crystal Violet, Point A, Flat, and 60 seconds.
14. Rinse with water at point B with a tilted orientation for 10 seconds.
15. Add Gram's Iodine to point A with a flat orientation for 120 seconds.
16. Rinse with water at point B with a tilted orientation for 10 seconds.
17. Add Decolorizer at point B with a tilted orientation for 10 seconds.
18. Rinse with water at point B with a tilted orientation for 10 seconds.
19. Add Safranin at point A with a flat orientation for 60 seconds.
20. Rinse with water at point B with a tilted orientation for 10 seconds.
21. Click the "Blot Dry" button to blot dry the smear.
22. Click the "Done" button to correctly store the smear.
23. Click the "Smear Examination" button.
24. Click the "Select Slide" button and select your slide.
25. You can use the forward and backward arrows next to the "Field" indicator to move between fields.
26. Once you have identified the shape of the bacteria and whether it is Gram-positive or Gram-negative, click the "Identification" button and select your answers.
27. Click the "Evaluation" button to see a rating of your performance.

### Do You Understand?

1. What is the purpose of the Gram staining procedure?
2. Does the order of operations matter in the Gram staining procedure?