

Student Guide: Gram Staining

Use the following set of instructions to stain your cultured bacteria, prepare a microscope slide and view it under a microscope. Write down your observations and explain how they compare with your prediction about the color of the Gram's stain for each type of bacteria.

Procedure:

1. Fill a 15-ml plastic test tube with 5 ml of water, add a drop of yogurt and put the cap on the tube. Seal the tube tightly, and shake and swirl it vigorously to mix it well.
2. Use a clean inoculation needle to put a drop from the tube on a clean microscope slide. If necessary, use the needle or the edge of another clean slide to smear the liquid into a very thin film.
3. Let the bacteria on the slide air dry for about two minutes, then hold the slide with forceps (tweezers) and move it back and forth through the alcohol lamp flame three to four times. Do not let the slide get too hot.
4. Put one to two drops of crystal violet stain on the sample and let it sit for 60 seconds.
5. Rinse off the stain with a gentle squirt from the water bottle.
6. Put one to three drops of Gram's iodine stain on the sample and let it sit for 60 seconds.
7. Rinse off the stain with a gentle squirt from the water bottle.
8. Tilt the slide and add ethyl alcohol one drop at a time so that the alcohol runs over the entire sample. Stop adding alcohol drops as soon as the liquid dripping off the slide becomes colorless. That might take about five seconds or so.
9. Rinse off the alcohol with a gentle squirt from the water bottle.
10. Put one to three drops of safranin stain on the sample and let it sit for 60 seconds.
11. Rinse off the stain with a gentle squirt from the water bottle.
12. Very gently blot (but do not rub) the slide with a paper towel to dry it.
13. Put a clean cover slip on the slide. If necessary, use another slide or a paper towel to very gently mash it down or move it into position. If instructed by your teacher, add a tiny drop of Canada balsam or microscope slide mounting cement before you put the cover slip on the slide.

14. Observe the slide under the microscope. Start at low power (40x), focus back and forth until you find a reddish-purple colored layer and then work your way up to higher powers, refocusing each time as necessary. (Be careful not to focus on other, non-colored layers that might just be dust, bubbles or scratches above or below the actual sample.)

15. Describe what bacterial shapes and colors you see. The bacteria will be tiny, even at 400x or 1000x magnification. You may also see clumps of protein, blobs of fat or other fairly large debris from the yogurt. If you see something photogenic, you can hold a cell phone camera up to the eyepiece and adjust the camera position and focus to take a photo.

Notes:

- If the bacteria are colorless, you may have used too little stain or left the stain on too briefly, or you may have rinsed with too much water or ethyl alcohol.
- If the bacteria are too dense, you can dilute a drop of yogurt with more water and make a new slide.
- If the bacteria are too scarce, you can dilute a drop of yogurt with less water and make a new slide.