



Video transcript

How to Make a Sponge Spicule Prep

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- In this video, I will show you how to make a sponge spicule prep using bleach.
- We are going to cut a small piece of sponge tissue, dissolve it in household bleach, rinse it, and look at it under the microscope.
- To get started, make sure you have a lab coat, gloves, and some kind of eye protection or eyeglasses.
- We are going to use household bleach and water. You will need a container to hold the waste bleach.
- I like making these spicule preps in a 24 well tissue tray.
- You will need some way to cut the sponge with a knife and some forceps.
- We will need some microscope slides and cover slips.
- And also some pipettes to remove the bleach and the spicules.
- I like using these pipettes where I cut the end off to make it a little shorter and with a wider end to the pipette in case you have some very large spicules.
- Make sure that you label the tray with some kind of number to identify each specimen.
- To avoid cross-contaminating each specimen, it is a good idea to also label the pipette tips used for each specimen.
- OK, let's get started.
- We will take our first sponge here, we will cut a small piece, and you do not need very much here, just maybe half a centimeter cubed.
- We will put this in the correct well, and then I have these labeled, though I always make a note of which one I'm working on.
- We will take this sponge, cut a small chunk of this sponge, place it in the well.
- We can then take our household bleach.
- And you just need to cover up the piece of sponge in each one of these wells.
- In this case, we are going to go about half full.
- Now this bleach will take about 30 minutes to one hour to work.
- It just depends on how big of a piece of sponge that you use, or the exact species of sponge you are using.



- After half an hour when we come back, we will see if they have digested.
- Now that the tissue has dissolved, we can remove the bleach.
- We just want to use a pipette and carefully suck up this bleach without disturbing the spicules underneath.
- And once you have removed the bleach, just add some water to each well.
- And you will want to let it sit for about 10 minutes for those spicules to settle down to the bottom of the well.
- Now we want to wash it again with water, so we are going to remove this water again, being careful not to disturb the spicules,
- And you should see a small film at the bottom of the well where the spicules are.
- Next, we just add more water. We will rinse the spicules 4 times.
- Once you have rinsed the spicules, we should see a whitish film at the bottom of the well.
- Now that we have rinsed the bleach off these spicules, we can put them on to a microscope slide.
- We are going to use some labeled pipettes to do that, one for each specimen so we do not contaminate the spicules of one species with those of another.
- And we suction off the bottom, and it's a good idea to hold the pipette still for a little bit, maybe 15 seconds, before you actually place it on the slide.
- Now, put a cover slip on each slide,
- And once you have a cover slip on the slides, we are ready to go look at these in the microscope.
- And this is our first sponge, and as I look at the slide, I can see mostly oxeas
- they have similar shape and length and width.
- Oxeas are basically small curved rods with a point on each end.
- Our second slide is *Iotrochota birotulata* and we can see very small birotules here.
- This is at a higher power, this is at 200x magnification.
- If I go to the previous magnification, which is 100x, we can still see the birotules.
- This sponge also has a mix of strongyles and styles.
- In this video we made our spicule preps using just water as the mounting medium.
- If you want a more permanent spicule prep, you need to use a medium like Canada balsam or Permount.
- You can find protocols for doing this on our website.