# Slime mould care guide: Physarum polycephalum

*A plain, step-by-step guide to reviving, feeding and studying your Rootlab slime mould culture.*

## 1. Meet your slime mould

Physarum polycephalum is a slime mould, a strange and brilliant single organism that behaves like something far cleverer than it has any right to be. In the wild it lives in cool, damp, shady places like leaf litter and rotting wood. It has no brain and no nerves, yet it solves mazes, finds the shortest route between food sources, and builds transport networks that rival ones designed by engineers. In one well known study, researchers laid food out in the pattern of the cities around Tokyo and used light to mark barriers the mould avoids, and it grew a network that closely matched the real Tokyo rail map.

A few things that make it worth your bench space:

- It is one giant cell. In its active plasmodial form it is a single cell holding many nuclei, sometimes more than ten billion of them, all dividing in sync. It can grow larger than a dinner plate.
- It flows and pulses. It moves by streaming its insides back and forth through a network of vein-like channels, roughly once a minute, which you can watch under a cheap microscope.
- It remembers where it has been. As it hunts it lays down a slime trail and then avoids that ground, a kind of memory kept outside its body, so it does not waste effort re-searching dead ends.
- It has a genome full of surprises. At around 250 million bases it carries an estimated 34,000-plus genes, some of its light-sensing genes resemble bacterial ones, and some look most like those found in animals.
- Classifiers have argued over it for decades, placing it in the Amoebozoa, the Mycetozoa, or the old myxomycete grouping.

## 2. A few rules before you open the kit

This kit is imported into Australia under permit, and there are conditions you agree to by using it. In plain terms:

- It is for laboratory and hobby culturing only. Do not release it into the environment, and do not expose it to plants, humans, or animals other than recognised laboratory animals.
- Leave the labels on the product and make sure anyone else handling it knows these conditions too.
- It is an adults-only kit (18 and over). Children can take part under adult supervision.
- Use the kit within 45 days of receiving it, and always store the sclerotium in the dark.

Safety-wise, treat everything you culture as if it could carry other microbes, even though Physarum itself is not considered a health risk under normal handling. Wash your hands before and after, keep food and drink away from your work area, and clean up when you finish. When you are done with a culture or any material that touched it, sanitise it before disposal, either by autoclaving or by soaking it covered in a one-part-bleach-to-nine-parts-water solution for a couple of hours. This is not a beginner project. It rewards patience and clean technique, and it

punishes sloppiness with contamination or nothing growing at all.

## 3. What is in your kit

Your kit (the Rootlab Physarum polycephalum slime mould kit: https://www.rootlab.com.au/product/slime-mold-kit-physarum-polycephalum/) is built so you do not have to make growth medium from scratch. It contains:

- The sclerotium, your dormant slime mould, to be kept in the dark.
- Five pre-poured 2 percent non-nutrient water agar plates, ready to use.
- Five sterile empty plates for later transfers.
- Five sterile filter papers.
- Sterilised oats, kept sealed at room temperature.
- A 20 mL sterile water syringe.
- Five single-use needles for placing tiny, controlled drops of water.
- A scalpel with ten sterile blades.
- Tweezers.
- Ten pre-cut parafilm strips for sealing plates.
- A 100 mL bottle of 70 percent isopropyl alcohol for sanitising.

Because the plates are already poured, you can skip straight to culturing. If you ever want to make your own plates, the recipe is 2 grams of bacteriological agar stirred into 100 mL of distilled or deionised water, autoclaved for 20 minutes at 121 degrees C under 15 psi, then poured about 5 mm deep into sterile dishes and left to set. But with this kit you will not need to.

## 4. Working clean: the non-negotiables

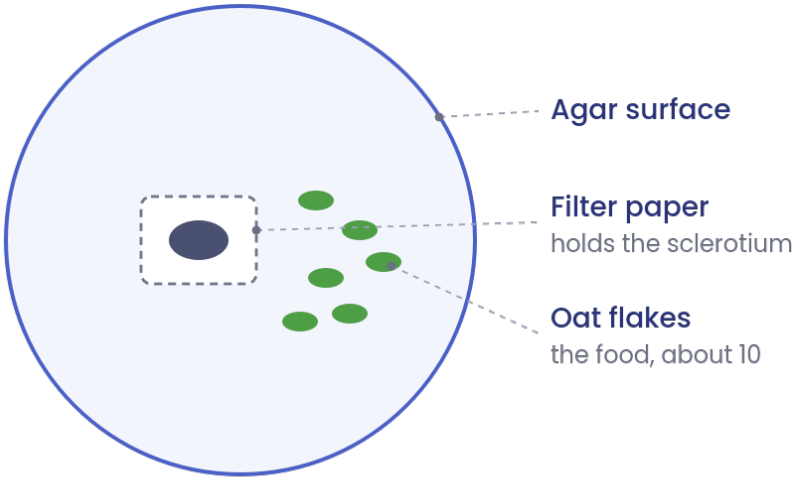
Contamination is the single biggest reason a culture fails, and it almost always comes from the air or from a tool you touched. Set yourself up properly:

1. Work in a completely still room. No fan, no air conditioning, no open door or window. Moving air carries spores.
2. Spray your bench and your hands with the 70 percent isopropyl before you start, and again when you finish.
3. Sanitise your tweezers and scalpel before every use, either by passing them briefly through a flame or by spraying with isopropyl and letting them sit for at least 20 seconds before touching anything.
4. Once a tool is clean, do not rest it on the bench or let it touch anything unsterile. Use it straight away.
5. When you take the cap off the water syringe, set it down on a clean surface only, spray it with isopropyl before putting it back, and store the syringe in a zip-lock bag at room temperature or in the fridge.
6. Keep the oat container shut except for the moment you are shaking flakes out, so the oats stay sterile.
7. Keep any plate open for the shortest time possible, and keep the lid held over the dish while you work in it.

8 Anything that has touched the slime mould goes to sanitised waste, never back onto your clean bench.

## 5. Waking it up and keeping it fed

This is the heart of it. You are going to rehydrate the dormant sclerotium so it revives into an active plasmodium, then keep that plasmodium fed and moving onto fresh plates. Here is how the plate should look when you set it up.




Seal with parafilm, keep dark, lid facing up

*Setting up the plate*

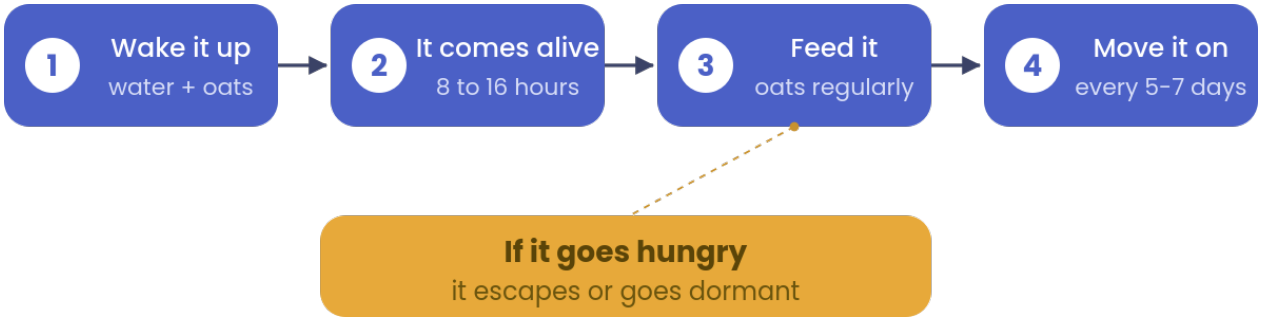
### Waking it from a sclerotium

1 Place one of the water agar plates in front of you. Using the syringe (a needle on the tip gives you finer control), put a single drop of sterile water in the centre of the agar. One drop only, too much water invites contamination.
2 Sanitise the tweezers, then use them to lay the sclerotium face-down onto that water drop.
3 Shake roughly 10 oat flakes from the container and arrange them around the sclerotium, then reseal the oats.
4 Put the lid on and seal the rim with a parafilm strip.
5 Sit the sealed plate somewhere dark with the lid facing up, a cupboard or drawer is perfect, and leave it for 8 to 16 hours.

Within that window the sclerotium revives into a plasmodium, which then flows off the filter paper onto the oats and out across the agar. From there it is all about feeding.

### Keeping it alive

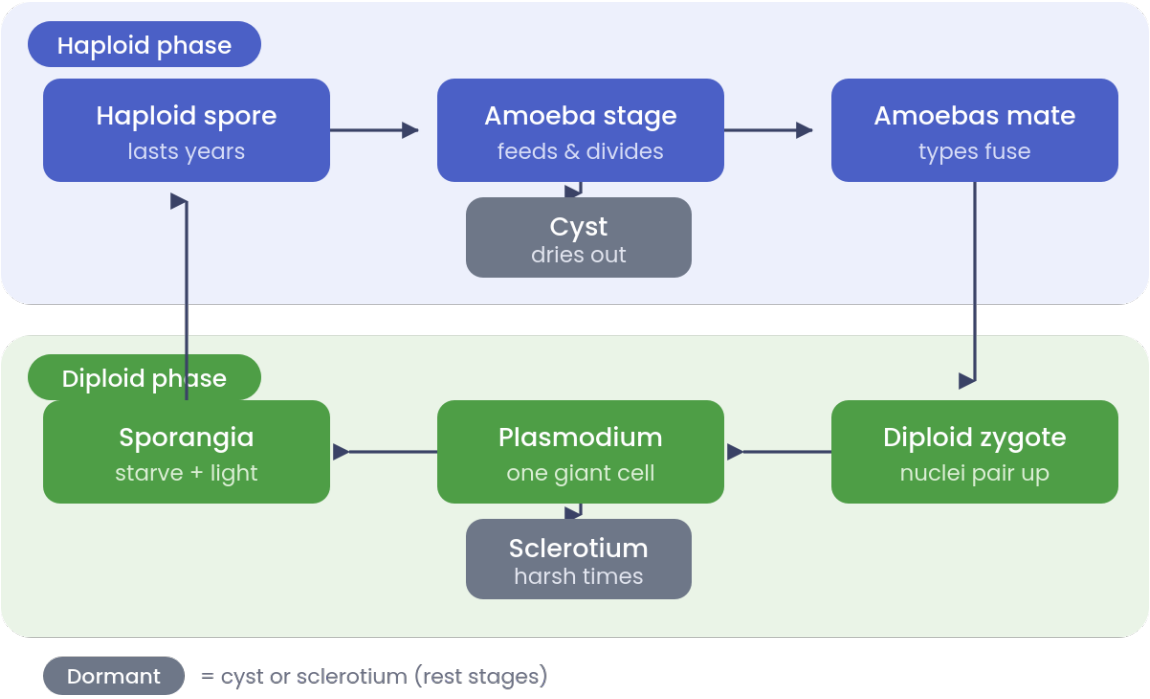
1 Keep adding a few fresh oat flakes as it grows. A well-fed plasmodium is a happy, spreading plasmodium.
2 Watch for hunger. If it runs low on food it will try to climb out of the dish, and if it gets dry or starved it will either make spores or retreat back into a sclerotium.
3 When it has spread across the whole plate, move it onto a fresh plate. As a rule, re-plate when it fills the dish or roughly every 5 to 7 days, whichever comes first.




*The ongoing care routine*

## 6. What is happening inside the dish: the life cycle

Now that you have watched it wake up, feed, and spread, here is the bigger picture your plate is a small window into. Physarum loops through several very different body forms, switching depending on moisture, food, light and stress. Some forms carry a single set of chromosomes (haploid) and some carry two (diploid), and there are dormant forms that are just shelters for hard times.




*The Physarum life cycle*

Walking the loop: a spore is a tough haploid capsule that survives for years. When conditions are damp enough it germinates and a single haploid amoeba crawls out. This amoeba eats bacteria and other microbes by engulfing them, crawls about, and multiplies by mitosis, splitting into two cells each time. In wet conditions it can grow whip-like tails and swim as a flagellate, and when things turn dry or nasty it walls itself up as a cyst until conditions improve. These switches are reversible, and that flexibility is exactly what lets it ride out a wide range of conditions.

Two compatible amoebas can then fuse into a single diploid cell, the zygote, with one combined nucleus. Which amoebas can pair up is governed by several mating genes, and pairs carrying different versions tend to fuse more readily than matching ones. In a neat twist, some strains skip the mating step entirely and a lone haploid amoeba grows straight into a plasmodium, a shortcut

called apogamy.

The zygote grows into the plasmodium, the giant, streaming, many-nucleated form you keep on your plates. Starve that plasmodium and hit it with light and it sporulates: bumps rise, grow into stalked sporangia, and make fresh spores, and the loop closes. Starve it in darkness instead and it takes the other exit, congealing into a sclerotium, a cluster of hard-walled resting cells that can sit for months and then revive. Spores can wait even longer, for years, before hatching a new amoeba.

## 7. Changing its state on purpose

Once you are comfortable keeping a plasmodium alive, you can deliberately push it into its resting or spore-making states.

### Making it form a sclerotium (banking it for later)

1. Lay a small piece of sterile filter paper (a quarter of one is fine) on a fresh agar plate, moisten it with a little sterile water, then lightly sprinkle sterile oats over it.
2. Using the transfer method above, cut a 1 cm square of plasmodium-covered agar and place it plasmodium-side-down on the filter paper. Seal with parafilm.
3. Let it grow until it covers the whole small filter paper.
4. Meanwhile, set up a larger piece of sterile filter paper inside an empty plate.
5. When the small paper is fully covered, moisten the larger paper with sterile water.
6. With sanitised tweezers, move the small plasmodium-covered paper, oats and all, to the centre of the larger paper.
7. Let the plasmodium spread across most of the larger paper, then remove the small paper with the oats so it starts to starve.
8. Slowly dry the larger paper out by leaving the lid slightly ajar. As it dries, a sclerotium forms, which you can store in the dark for next time.

### Making it sporulate

1. Let a plasmodium fill its plate and completely run out of food.
2. Turn the plate upside down somewhere that gets daylight, but not right beside a window in direct sun.
3. Wait. It may take several days or longer. If nothing has happened within two weeks, it probably will not.

Sporulation is genuinely fiddly, it is normally done under tightly controlled lab conditions, so treat any success here as a win rather than a given.

## 8. Getting a closer look

A stereomicroscope at 10x to 40x is the ideal way to view it, with light coming from below. The back-and-forth streaming is easiest to catch on an active plate during growth, especially in the vein-like strands near the advancing edge, and 40x reveals far more detail than 10x. If you only have a compound microscope, use its lowest, scanning objective (keep total magnification at 40x or under), open the diaphragm for maximum light, and keep the lid on to protect the lenses.

To view a plasmodium on a compound scope, grow a thin film on a slide:

1. Soak a standard slide in the isopropyl for at least an hour, then let it dry, covered, in a clean plate, ideally overnight.
2. Coat the top of the slide with a thin layer of sterile agar and let it set, keeping it covered.
3. Seed it with a small (about 0.5 cm) chunk of plasmodium-covered agar, plasmodium-side-down, and keep the slide in a closed plate the whole time. Add a drop of sterile water if it starts to dry.
4. The plasmodium usually creeps onto the thin agar within about a day, ready to view.

With the naked eye, spores look like tiny dark poppy seeds. Under magnification you can see their lobed structure.

## 9. Things to try

Once your culture is established, it is a superb little experimental subject. Some ideas:

1. Set it a maze. Cut a maze from a sheet of acetate, sit it on an agar plate, sanitise it, and place food at two points. See whether the plasmodium finds the shortest path between them. It may occasionally cross the acetate, so try different materials or coatings to stop shortcuts.
2. Test what attracts and repels it. It tends to move towards some sugars (glucose, galactose, mannose) and away from others (sucrose, ribose). Try herbs and oils too, such as clove, cedar, basil or geranium, run each test a few times, and compare.
3. Play with temperature and see how the plasmodial form responds.
4. Test its memory. Let it lay down a slime trail, then see whether the same or a different plasmodium avoids that ground when foraging later.
5. Trigger a sclerotium different ways. Compare drying, cold, heat, salty or acidic solutions, and starvation in the dark, and see which brings it on fastest.

And a few questions worth chewing on while you watch it:

- Physarum carries more cargo-hauling kinesin proteins than its close relatives. Given that it is one enormous cell rather than many small ones, why might that be? A sensible answer: material has to travel much further inside a single giant cell, so it pays to devote more machinery to moving things around.
- Its slime-trail memory saves it from re-searching ground it has already covered. What is the advantage of that, and what other creatures use chemical trails while foraging? Ants are the obvious example.
- Its classification has shifted over the years. Pick a point in time, and argue for placing it in a particular group based only on what was known then. There is no single right answer, the reasoning is the point.