



SLIME MOLD culture kit

Manual



## Contents page

Glossary .....	2
Kit description and usage .....	2
Best environment for cultures .....	3
Life cycle .....	4
Starting a culture .....	6
Making your substrate .....	8
Transferring a plasmodium .....	11
Making sclerotia .....	12
Where to find oat flakes and agar-agar in retail stores .....	14
Known issues and solutions .....	15
Precautions .....	16

## Glossary

This part is aimed at defining technical terms you'll encounter in this manual for the sake of clarity.

**Petri dish:** It's a round translucent plastic box with a lid. Its main purpose is to contain and grow cultures.

**Slime mold:** Also known by its species name: *Physarum Polycephalum*. It's a single cell visible to the naked eye and looking like yellow colored mold.

**Agar-agar:** An element found in some species of red algae. When put in water and heated close to boiling temperature, it turns into a vegetal jelly after cooling down.

**Substrate:** It's a culture medium. In this manual, the substrate will be a jelly made of 100ml of water for each gram of agar-agar. It will allow slime molds to grow on it while keeping them moist.

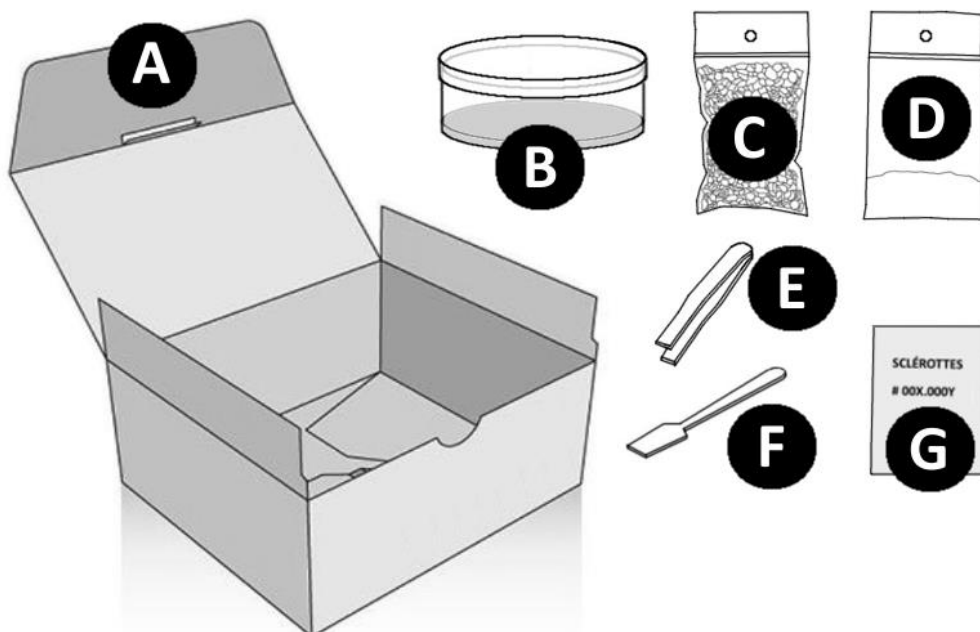
**Plasmodium:** Awoken slime mold that can spread, move and feed.

**Sclerotium:** Dehydrated form of a plasmodium that looks like a yellow crust. This state allows plasmodia to preserve themselves from hostile environments.

## Kit description and usage

This kit is aimed at growing slime molds. This species is a cell visible to the naked eye that shares some properties with mushrooms, plants and animals but does not belong to them. By following the instructions given in this manual, you will be able to wake up sclerotia to get plasmodia and to grow them in petri dishes in the best conditions.

This kit is intended for those who have curiosity and interest in scientific entertainment and aged 8 and more.



**A) Cardboard box**

- Allows you to cover your cultures to keep them away from day light. You may also put them in another place such as a drawer or an opaque box.

**B) 2 petri dishes with 1 containing prepared substrate**

- To store your slime mold cultures (refer to page 6 « Starting a culture »).

**C) 2 bags containing 17g of oat flakes**

- Used to feed your cultures.

**D) 1 bag containing 4g of agar-agar**

- To make your own substrate (refer to page 8 « Making your substrate »).

**E) 1 plastic tweezers**

- Helps you to take oat flakes or culture parts (refer to pages 6 « Starting a culture » and 11 « Transferring a plasmodium »).

**F) 1 plastic spatula**

- Helps you to take culture parts (refer to page 12 « Making sclerotia »).

**G) 4 sclerotia on paper**

- To start a culture (refer to page 6 « Starting a culture »). Sclerotia are contained in a petri dish, which is labeled with a root culture number (to be indicated to customer service if you contact them).

### **Best environment for cultures**

Slime molds can be found in nature, in dark and moist areas (undergrowth, under some trees barks, etc.). Therefore, slime molds prefer humidity and dark places to grow better. However, you might light your cultures while you look after them.

When you don't use your cultures, you should put them in darkness. For instance, you might turn the kit's box and cover your cultures. You may also place them in a drawer, cupboard or any other dark place.

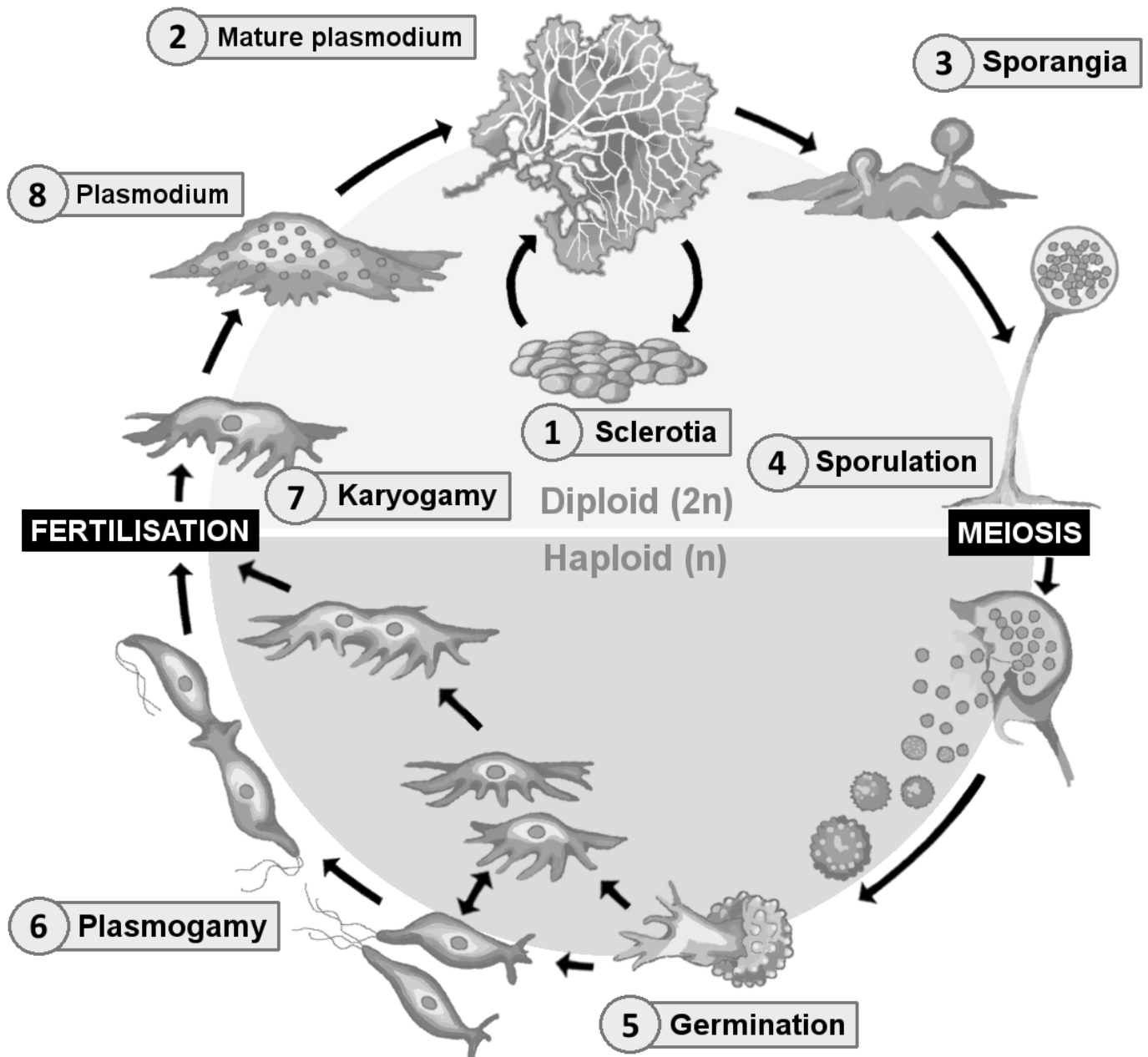
Slime molds feed on mushroom spores, bacteria and other microorganisms in nature. However, they can feed on bacteria living on oat flakes. Those given in the kit are sufficient to feed them more than a month. Oat flakes are easy to find in retail stores if you need more of them.

Even if slime molds need humidity, your substrate will be enough for its growth. It's not recommended to place your cultures in a moist room, for your oat flakes could go moldy quicker.

Ambient temperature of the room hosting your cultures should be from 10°C to 25°C (50°F to 77°F) to ensure a good growth of your slime molds.

## Life cycle

During its life, a slime mold can enter several states according to its environment. These are detailed below:



(1) **Sclerotium**: Dehydrated slime mold. When its environment becomes hostile (too dry, too cold, etc.). Its color is yellow, sometimes dark yellow, dry and rough to the touch. In this state, it can be stored in a dry and dark place for several years as long as it doesn't lack oxygen.

(2) **Mature plasmodium**: Multinucleated cell. It can move to feed on nearby food sources. It spreads a vein-like network named pseudopodia. This kit is aimed at cultivating slime molds under this form.

(3) **Sporangia**: When a plasmodium lacks food while being exposed to light, it starts growing dark balls containing spores. In nature, it allows a slime mold to scatter its

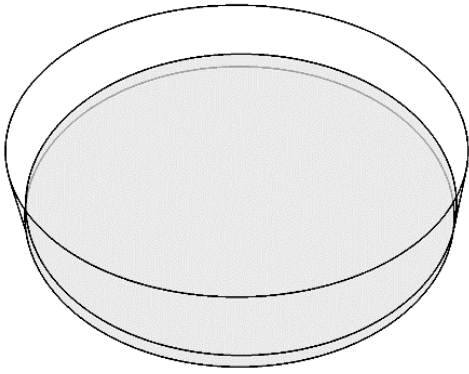
spores when local food sources have been depleted. It is not advised to attempt to reach this state while using this kit, for spores might be volatile and even though they pose no health risks, they might stain the close surroundings of your cultures.

- (4) **Sporulation:** Every sporangium starts making spores by meiosis (equivalent to sperm and ovum in other organisms). Once mature, sporangia release their spores in the air.
- (5) **Germination:** Spores germinate to give haploid cells (that contain in their kernel only one version of each chromosome). They can differentiate into ameba or flagellated gametes. Ameba can turn into flagellated gametes, and conversely.
- (6) **Plasmogamy:** Ameba and flagellated gametes' cytoplasm (material within the cell) might fuse to enter fertilization phase (karyogamy).
- (7) **Karyogamy:** Cell kernels fuse to make an egg cell (zygote), which is diploid (that contain in its kernel two versions of each chromosome).
- (8) **Plasmodium:** Cell kernels multiply and spread to give a mature plasmodium seen in (2).

This kit is designed to cultivate plasmodia and making / waking up sclerotia.

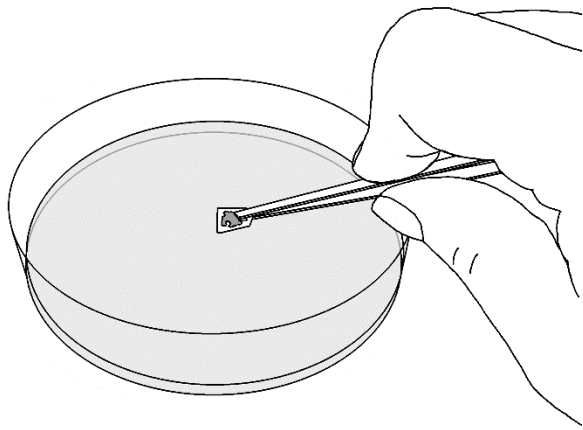
## Starting a culture

**1) To start a culture, you can use the prepared petri dish given in this kit.**



*If you'd rather start using an empty petri dish, you may produce your own substrate (refer to page 8 « Making your substrate ») and cast it on an empty petri dish or any other heat and moisture resistant container.*

**2) Take a sclerotium from its petri dish (yellow crust on a paper) and lay it on the substrate, at the center of petri dish. Yellow crust must be directed upwards.**

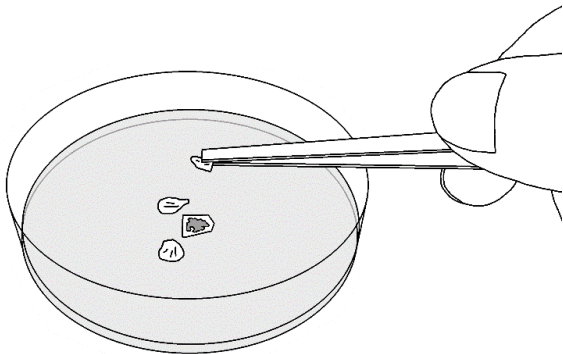


*It's also possible to wake up this way sclerotia you made (refer to page 12 « Making sclerotia »).*

*If you start a culture from a sclerotium, substrate moisture will wake up the sclerotium and keep it hydrated once it turned into a plasmodium.*

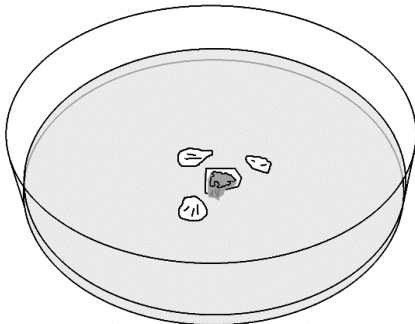
*You might also start a new culture using a plasmodium from another culture (refer to page 11 « Transferring a plasmodium »).*

**3) Using tweezers, lay 3 to 4 oat flakes a few millimeters away from the sclerotium.**



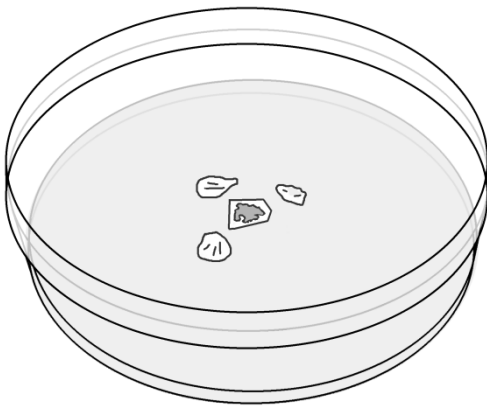
*Be careful not to put any oat flake on the sclerotium or it might suffocate.*

**4) Look after your culture once or twice a day minimum to monitor its growth.**



*Sclerotia can take from 12 to 72 hours to wake up and turn into plasmodia. If a sclerotium doesn't wake up after such period, you might contact our customer service for help.*

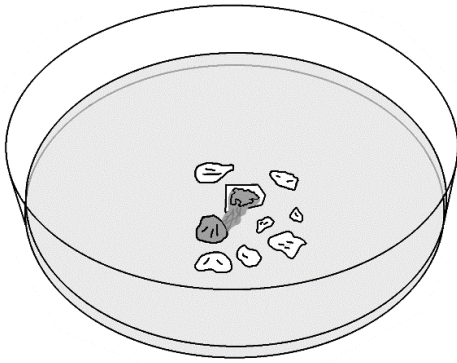




**5) IMPORTANT: Put back the petri dish's lid when you don't look after a culture.**

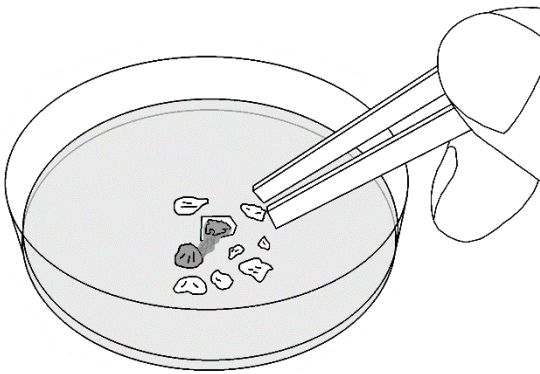
*The substrate is essentially made of water and dries little by little.*

*Closing your petri dishes allows substrates to keep their moisture longer and thus increases the life span of your cultures.*



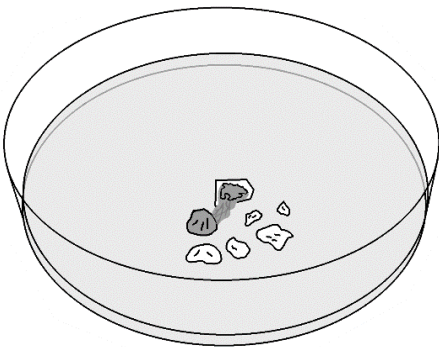
**6) Once a plasmodium reaches 1 or several surrounding oat flakes, you can add more of them near its advancing area.**

*You can notice a plasmodium reached an oat flake when the flake turned yellow.*



**7) Remove oat flakes the plasmodium left or if it takes more than 24 hours to reach them. It helps limiting any molding contamination.**

*A plasmodium can move at about 1cm / h and even 4cm / h if it's hungry. These are average speeds and variations might be observed depending on the culture.*



## Making your substrate

Petri dishes given in this kit already have a substrate.

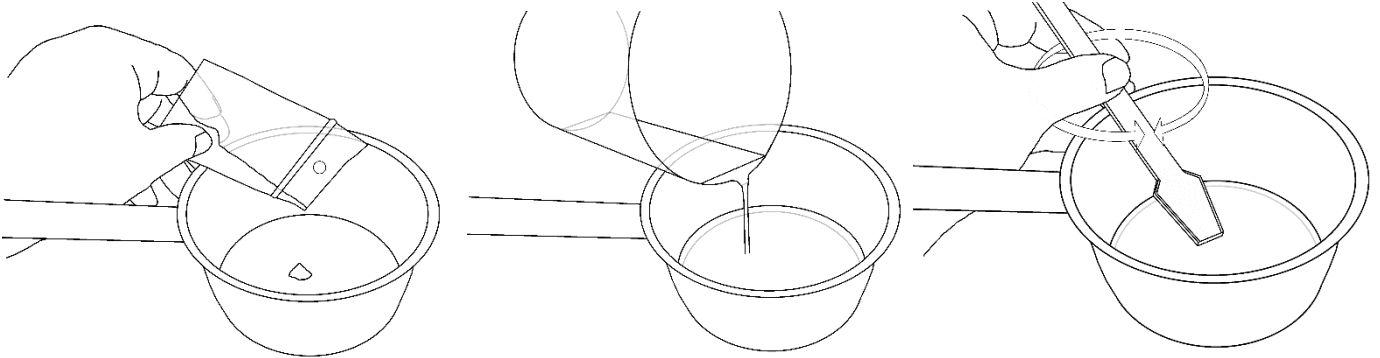
However, a bag containing 4g of agar-agar is also available to allow you to make about 400ml of new substrate (that corresponds to about 20 petri dishes).

*Indication: 1 teaspoon is about 1g of agar-agar and 1 tablespoon is about 2g of agar-agar.*

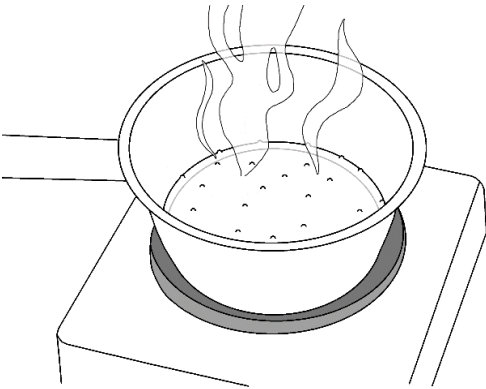
Below are instructions to make your substrate in 2 steps:

### A) Preparing a substrate

- Using a pan:

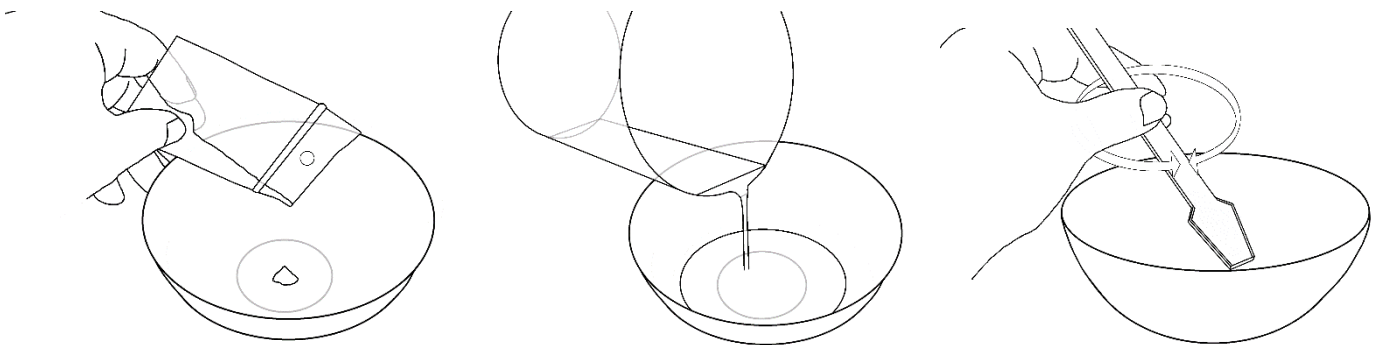


- 1) Mix agar-agar and water in a pan, given that you need 100ml of water for every gram of agar-agar, then start heating it.

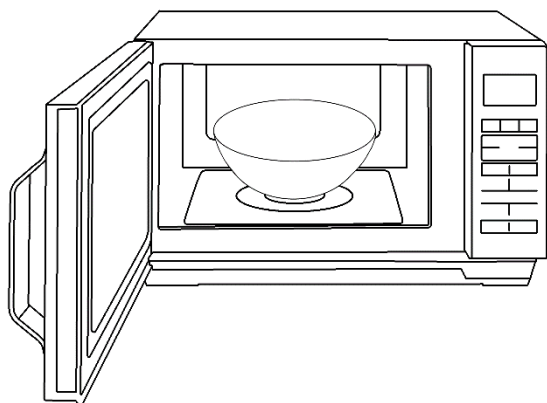


- 2) When it starts boiling, remove the pan from heat source (careful not to get burnt).

- OR using a microwave oven:



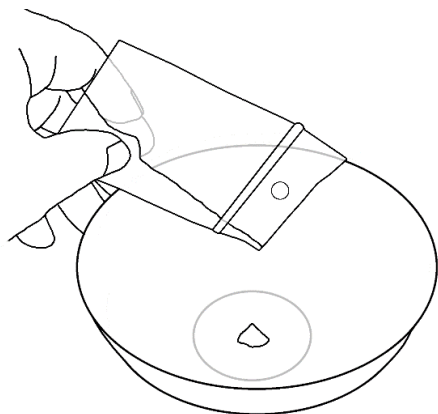
- 1) Mix agar-agar and water in a heat and moisture resistant container that is compatible with your microwave oven, given that you need 100ml of water for every gram of agar-agar.



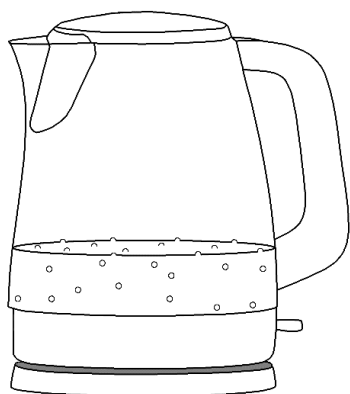
- 2) When it starts boiling, remove the container from microwave oven carefully.

*Approximately, it needs a microwave oven 30 seconds at 900 W to heat 50ml of substrate (equivalent to a small tea cup).*

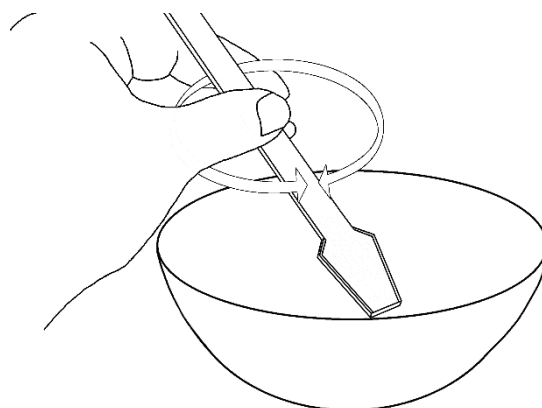
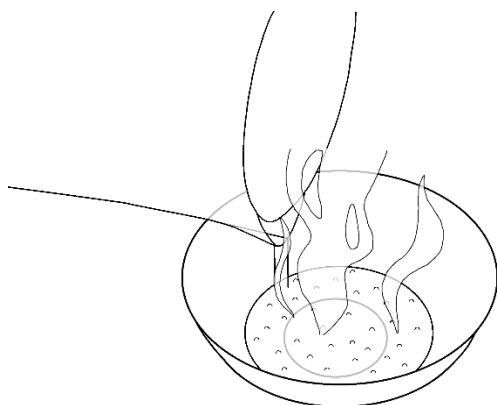
- OR using a kettle:



- 1) Set the needed quantity of agar-agar in a heat and moisture resistant container.

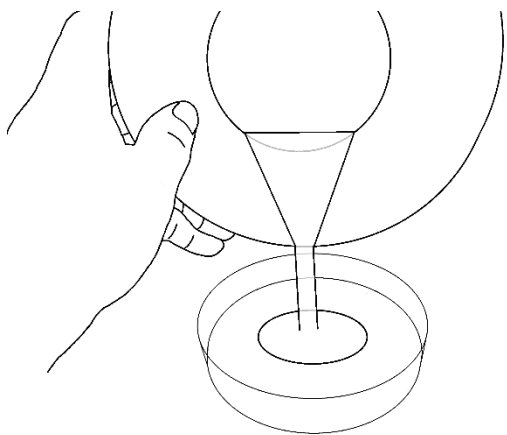


- 2) Boil water in the kettle, given that you need 100ml of water for every gram of agar-agar.

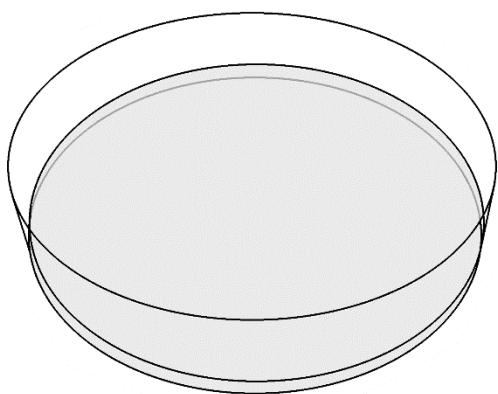


3) Put the hot water in the container and mix it with agar-agar.

## B) Pouring the substrate



1) Pour the substrate while it's still hot in your petri dish or any heat and moisture resistant container (height of substrate layer should be about 3 to 5 millimeters).



2) Set the petri dish or container with substrate horizontally on a heat and moisture resistant surface. Let it cool down for about 15 minutes.

*Every unused substrate can be poured in a closed heat and moisture resistant container and left in a fridge for a duration up to 3 days.*

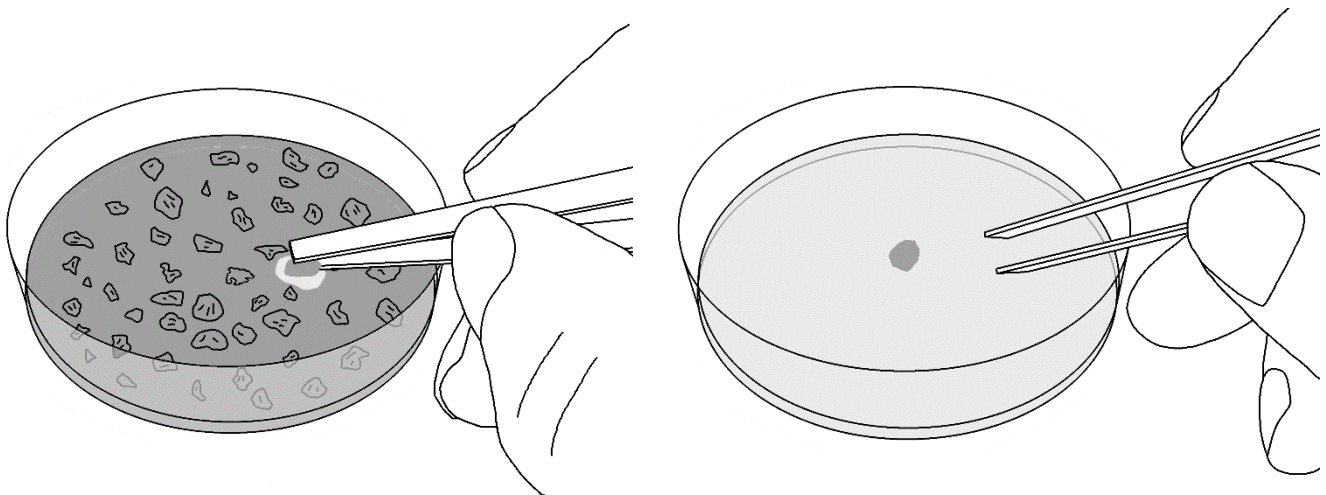
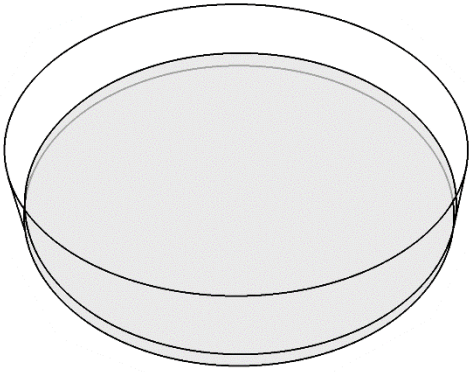
*It's better not to pour hot substrate in a container such as a bottle, for it will make a jelly while cooling down that might be difficult to get out from the bottle.*

*You may melt again unused substrate in a microwave oven or a pan. Once it became liquid, you can pour it in an adapted container and it will jellyfy again while cooling down.*

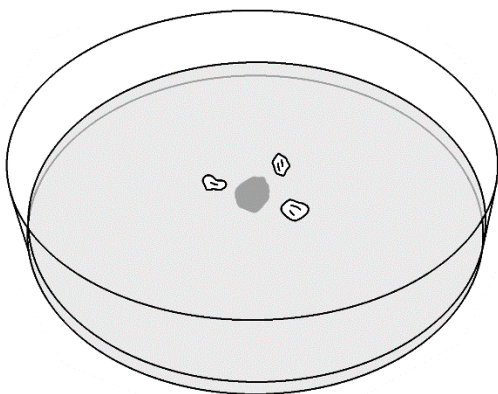
## Transferring a plasmodium

You might start a new culture by adding a part of plasmodium from another culture. Here is how to proceed:

- 1) Take an already prepared petri dish (or containing a substrate of your own).



- 2) Take a plasmodium part from the existing culture (about 0,5 cm<sup>2</sup>) using the tweezers and lay it on the new petri dish's substrate.



- 3) Put oat flakes around the plasmodium in the same fashion you would do it for a sclerotium (refer to page 6 « Starting a culture »).

*It's perfectly possible to add a plasmodium to an existing culture to attempt to increase its growth speed.*

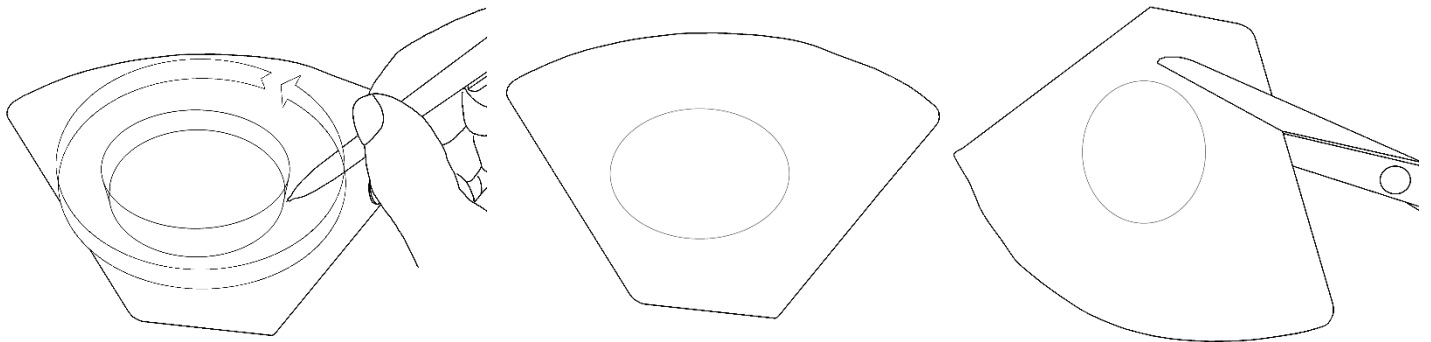
*Adding a plasmodium which is over an oat flake from another culture is not recommended, as it might increase your chances of having mold contamination.*

## Making sclerotia

To produce your own sclerotia, you need to make your culture environment hostile in a controlled manner. Here is how to proceed:

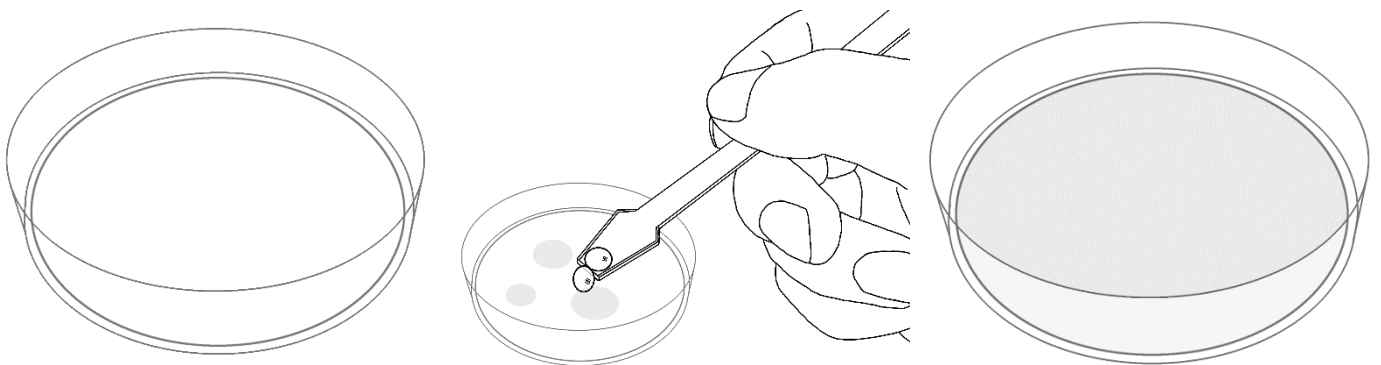
You will need the following elements:

- A culture containing a plasmodium.
- A petri dish or any other moisture resistant container.
- A lid to cover the container (plastic film if no lid is available will do the trick).
- Filter paper, pen and scissors.
- Water.



- 1) Take a filter paper (for instance a coffee filter). Cut it so that it fits in your empty petri dish.**

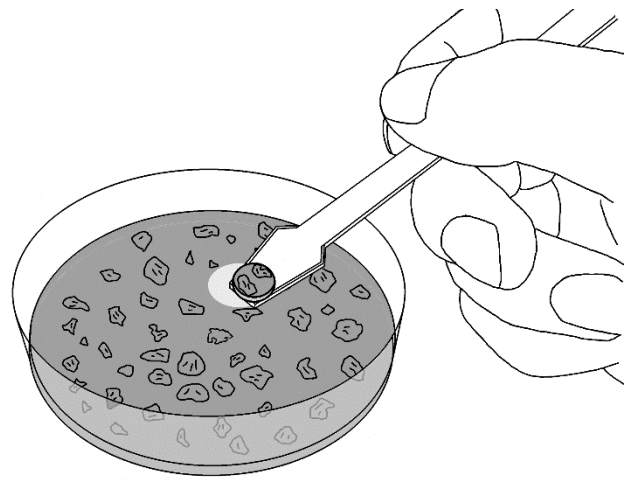
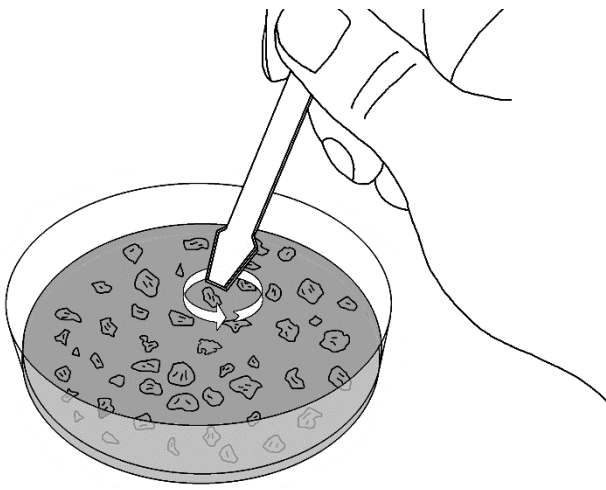
*You might use a pen and draw around your petri dish to have a round of the right size. Then, cut the round of filter paper with your scissors.*



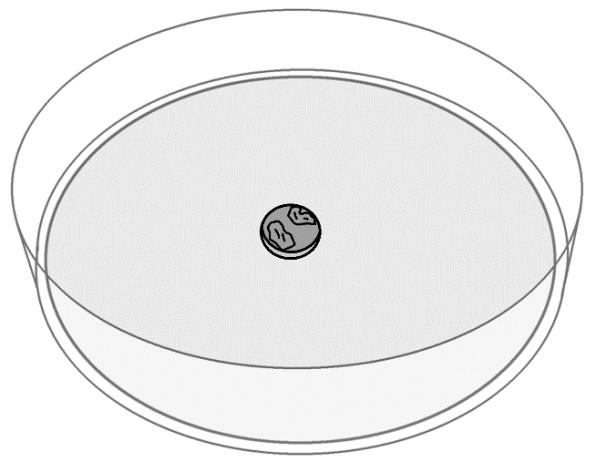
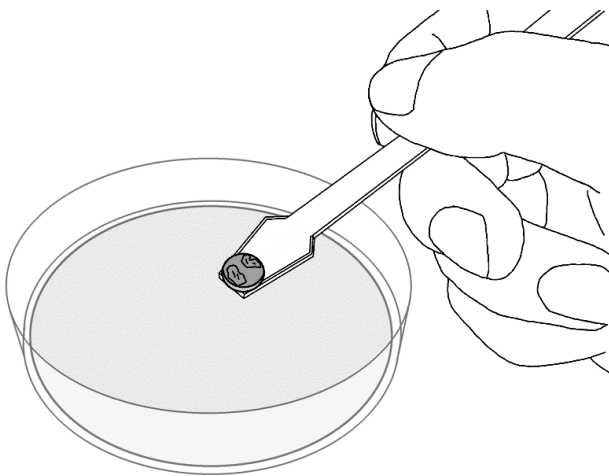
- 2) Lay it on the bottom of your petri dish and wet it using the spatula. Every part of the filter paper must be wet but not drowned in water (no level of water over it).**

*You might use the spatula to lay drops of water until the filter paper is uniformly wet.*

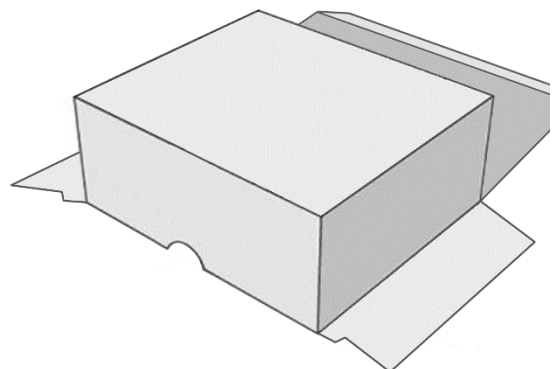
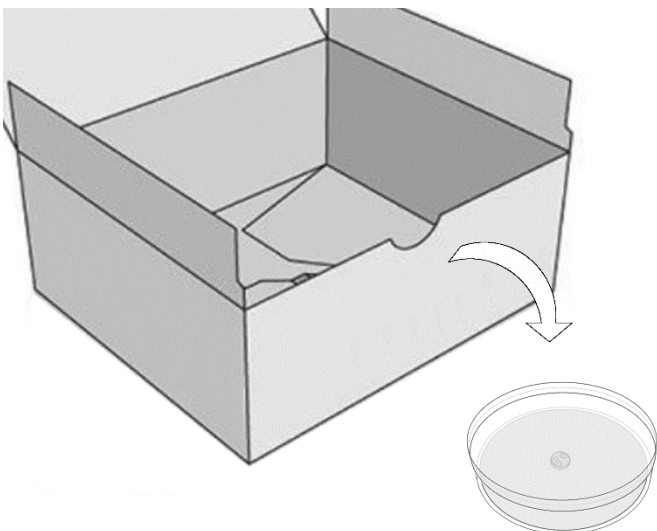




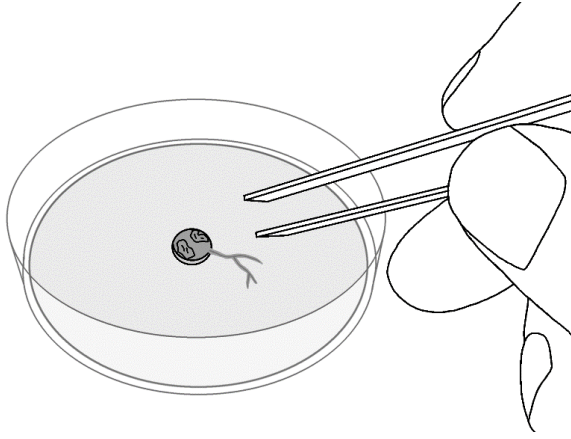
**3) Using the spatula, cut a part of about 1 cm<sup>2</sup> in the existing culture. Then take it using either the spatula or the tweezers.**



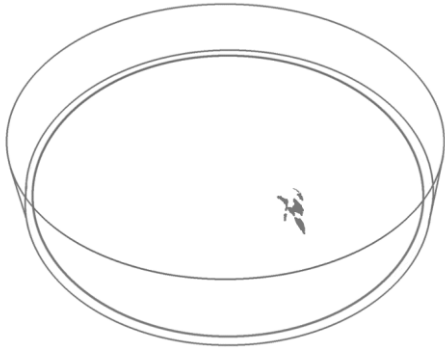
**4) Gently lay the culture part you took in the center of the petri dish, over the now wet filter paper.**



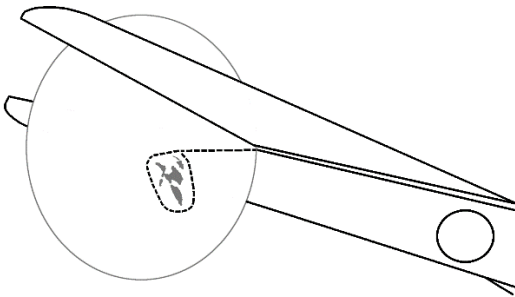
**5) Close the petri dish with its lid and put it in a dark place at ambient temperature.**



6) After about 24 hours, look after your petri dish. If you see that the plasmodium left its former substrate (the part you previously extracted from the existing culture) and if it went on the filter paper, remove the former substrate using tweezers and get rid of it.



7) Don't close the petri dish with its lid this time and simply let the filter paper dry in the petri dish in a dark place at ambient temperature for about a week.



8) You may now cut the filter paper around your new sclerotium.

*Please note that you can do several attempts at once in case your plasmodium doesn't dry into a sclerotium.*

## Where to find oat flakes and agar-agar in retail stores

In case you used all of the oat flakes and agar-agar given in this kit, you may easily find them in a retail store or a supermarket.

### Oat flakes:

- They don't need to be organic.
- They are usually contained in 500g bags and cost approximately 2 € (1.80 £).

### Agar-agar:

- You may find it in pastry section of retail stores or supermarkets.
- It's often set in a box of 6 bags of 2g each and such a box costs about 3 € (2.70 £).
- You might find a bag of 500g online for about 40 € (36 £).



## Known issues and solutions

While experimenting with your cultures, you may come across some issues. Here are solutions to help you to solve them efficiently:

### 1) Substrate getting dry

The substrate being mainly made of water, it dries as time is going on, leading to a dried film. Any plasmodium on a dry substrate will retract and start to turn into a sclerotium, for its environment doesn't allow it to grow further.

#### **Solution:**

It is necessary to make a new substrate in another petri dish (refer to page 8 « Making your substrate ») and to move the plasmodium on the drying substrate to the new substrate (refer to page 11 « Transferring a plasmodium »). It is advised to close your cultures with a lid when you're not manipulating them to slow down the drying of their substrate.

### 2) Mold contamination

Plasmodia don't eat oat flakes, but the biofilm made up of microorganisms growing on them. Therefore, these oat flakes deteriorate in contact with water and end up molding. In the long term, it might kill a culture if molding spreads too much. Molds might take different colors: white, green, brown, black... In any case, it can cover plasmodia while spreading.

#### **Solution:**

If molds spread across the whole culture, you might recover a part of plasmodium or sclerotium if it's still visible. Using tweezers, you can take it and attempt to transfer it to a new substrate in another petri dish. Though be careful not to take any mold at the same time to avoid further contamination (refer to page 11 « Transferring a plasmodium »).

If molds are limited to a part of your culture, you might extract it using the spatula or a rounded knife to get rid of it (you might use cleaning paper to help you). Then, you may pour a hot substrate in the hole made by the extraction. Once this new substrate cooled down and became solid, you may lay new oat flakes to feed your culture. Be careful that every mold has been removed to prevent further contamination.

### 3) Plasmodium growing on the sides and outside of its container

While looking for food sources, a plasmodium spreads. After a given time, it might climb the sides of its container and even under the lid.

In some cases, a culture might get out of its container. In such situations, the plasmodium doesn't go far unless the surroundings of the container are moist.

**Solution:**

To avoid any escape, it's recommended to close back your container with its lid when you're done manipulating it. It's advised to look after your cultures at least once a day to anticipate such events. If you notice that a plasmodium is starting to climb on the sides of its container, you may remove the climbing part using the spatula and cleaning paper before getting rid of it. Instead of throwing it away, you may also attempt to start a new culture using only the spatula or tweezers (refer to page 11 « Transferring a plasmodium »).

**4) Molds appearing as spots on the substrate**

If your substrate, once prepared, stays several weeks without being used, mold spots might start appearing on it.

**Solution:**

Molding contamination of your culture increases a bit with such a substrate. You can make a new substrate if you wish, after getting rid of the old, contaminated one (refer to page 8 « Making your substrate »).

## **Precautions**

Please do not eat any content of the kit. Even though slime molds are harmless, it's not advised to ingest them, nor oat flakes, nor any element of your cultures.

Be careful not to put slime mold on your fabric (clothes, etc.) for it stains and might be difficult to clean. Using the spatula and the tweezers, or even cleaning paper is advised instead of direct skin contact with a culture.

You should look after your cultures at least once a day to anticipate possible issues before it gets too bad (molding, drying substrate, etc.).

Water used for your cultures might come from a tap, as long as it's clean enough not to contain pathogenic germs (most of domestic tap water is fine to be used).

It's advised to close your petri dishes or other containers properly with their lids to prevent drying substrates.

Please handle liquid substrates with caution, as they're still hot. There might be a risk of burning if they come in contact with skin while being still hot.

You should use a heat and moisture resistant container while manipulating hot substrate.

If you use a microwave oven to heat a substrate, please do not put a culture in the microwave oven. Excessive heat might kill plasmodia and liquid substrate might drown them. Instead, melt your substrate in a heat and moisture resistant container which is compatible with your microwave oven, then pour this new substrate in your culture container.

It's advised not to pour a new substrate over an existing culture (for it might smother the plasmodium). However, you can pour it in an available place in your culture (for instance, if you removed a part of it and left a hole) or in an empty petri dish.

If you're willing to get rid of a substrate surplus, please do not pour it in a sink, shower, toilets, etc. It might clog your pipes. You might put it instead in a bin used for organic waste once the substrate is cool enough to be solid.

If you wish to use a bigger culture container (for instance a tupperware), make sure that it's heat and moisture resistant before starting your new culture.

Any minor child must manipulate this kit under the supervision of a responsible adult.

Slime molds being living organisms, variations in growth and resilience might be observed amongst different specimen.

Bluedot.tech (SAS) declines any liability in case of problems caused by the usage of this kit that hasn't been planned in this manual.

If you experience any difficulty while using this kit, you might contact our customer service for help in our website.

Possibilities given by this kit broaden as we experiment them. You'll find the latest version of this manual in our website:

<http://blob.bluedot-tech.fr>



We wish you pleasant experiments!

