

## Preparation of a Small Bag with Substrate

This protocol explains how to prepare a sterile small bag to expand the amount of mycelium with the goal of fructification, fabrication or to use it as inoculant for bigger vessels.

**Materials for 1 bag:** Bucket that can hold 5 L (for mixing), jar with clean grain spawn, substrate, alcohol 70%, autoclavable plastic bags, gloves, distilled water (approx. 2x the dry weight of the substrate).



| Overall composition |       |
|---------------------|-------|
| Total dry weight    | 200 g |
| Grain spawn         | 15%   |
| Gypsum              | 2%    |

| Ingredient   | Sub. Composition | Weight       | Custom quantity |  |
|--------------|------------------|--------------|-----------------|--|
|              |                  |              | Multiplier      |  |
| Hemp         | 70%              | 116 g        | x               |  |
| Bran         | 30%              | 50 g         |                 |  |
| Spawn        |                  | 30 g         |                 |  |
| Gypsum       |                  | 4 g          |                 |  |
| <b>Total</b> | <b>100%</b>      | <b>200 g</b> |                 |  |

### Part 1: Weigh the Substrate

- Weigh the solid components and put them in the same bowl
- Add the distilled water
- Mix well, taking care of breaking down the chunks and mixing the wet substrate at the bottom
- Put the substrate in a filter bag (or an autoclavable container with air filter)



## Part 2: Sterilise

- Sterilise by pressure cooking the bag for 50 minutes
- When done, allow to cool down. It may take hours, therefore squeeze it to feel the temperature in the center



## Part 3: Mix In Mycelium

- If you are using grain spawn, break them down by shaking the jar, or take a sterile or flamed spoon and mix the grain in the jar
- If you are using liquid culture, with a sterile or flamed spoon mix your content well.
- Pour or spread the inoculant in the bag and seal the bag
- Let the bag roll to mix well the content



## Part 3: Incubate

- Incubate for 1 to 6 weeks in the dark; check every two to three days how well the mycelium is doing and if any contamination appears
- When the substrate is completely colonised, you can proceed with the next steps

