

## 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:** See below, bucket that can hold 5 L (for mixing), jar with clean grain spawn, substrate, alcohol, autoclavable plastic bags, gloves, distilled water (approx. 2,5x the dry weight of the substrate).



Overall composition	
Total dry weight	200 g
Grain spawn	35%
Gypsum	2%

Ingredient	Sub. Composition	Weight	Custom quantity	
			Multiplier	
Hemp	70%	88 g	x	
Bran	30%	38 g		
Spawn		70 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. This substrate can stay very hot for hours, therefore always 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 your liquid culture is not already fragmented, 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

