

INSTRUCTIONS

NANOSTABILIZER[®]-LSTM

USER GUIDE:

WITH BSP-1200 PROCESSOR IN THE FLOW-THROUGH CONFIGURATION



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MATERIALS NEEDED:

- BSP-1200 ultrasonic processor configured in the flow-through mode (see BSP-1200 User Manual and BSP-1200 Peripheral Equipment Assembly Guide for details).
- Digital scale, peristaltic pump, large-capacity in-line filter assembly with 1 micron cartridge, 7.5 L (2 gal) bucket (24 cm/9.5" diameter), immersion blender, silicone spatula, dark-glass presterilized finished product containers (enough to fit 20 L).
- NanoStabilizer®-LSTM, mushroom extract, distilled water.

INSTRUCTIONS FOR MAKING 20,000 ml (20 L) OF NANOEMULSION:

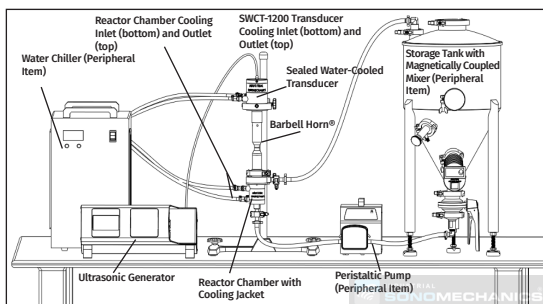
The instructions below detail the method for preparing 20,000 ml (20 L/20 kg) of nanoemulsion with the mushroom extract concentration of **50 mg/ml**. If a different concentration is desired*, use the table below and substitute the bolded numbers in the instructions with the numbers in the colored boxes.

Mushroom extract concentration in nanoemulsion**	30 mg/ml	40 mg/ml	50 mg/ml	60 mg/ml	70 mg/ml
Mushroom extract (kg)	0.6	0.8	1	1.2	1.4
NanoStabilizer®-LSTM (kg)	2.4	3.2	4	4.8	5.6
Distilled water (kg)	17	16	15	14	13
Total (kg)	20	20	20	20	20
Number of 50 mg doses of mushroom extract per 20 L/20 kg of nanoemulsion	12,000	16,000	20,000	24,000	28,000

* If your intention is to convert this nanoemulsion into a water-soluble powder, we recommend that you stay with the 50 mg/ml concentration, as detailed in this guide. We also recommend that you dry/powderize the nanoemulsion within 48 hours of producing it.

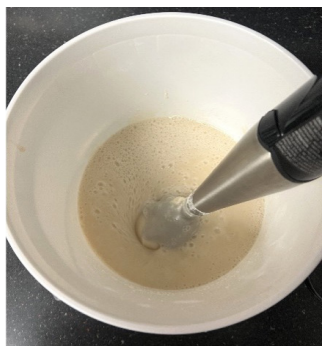
1 Setting up the liquid recirculation network, adding and heating distilled water:

- Assemble the BSP-1200 ultrasonic processor in the flow-through mode (see BSP-1200 User Manual, BSP 1200 Peripheral Equipment Assembly Guide, and schematic on the right for details).
- Make sure the bottom outlet valve of the storage tank with mixer is closed. Dispense 7 L of distilled water into the storage tank with mixer. Start running the mixer at a high speed (80–100%).
- Check that all the connections in the process recirculation network are properly clamped. The liquid should be set up to flow from the bottom of the storage tank with mixer to the reactor chamber with cooling jacket, and then back to the top of the storage tank with mixer (see BSP-1200 User Manual and BSP-1200 Peripheral Equipment Assembly Guide for details).
- Open the bottom outlet valve of the storage tank with mixer. Turn the pump ON, then immediately OFF and check for any leaks in the process recirculation network. For assurance, perform this step several times. If no leaks are observed, proceed to the next step.
- Turn the pump ON and begin recirculating the distilled water at a flow rate of 2–2.5 L/min (approximately 300 RPM).
- Turn the water chiller ON and verify that it is supplying cool water to the transducer's and reactor chamber's cooling jacket (see BSP-1200 User Manual for details). Turn the water chiller OFF. Disconnect the cooling lines from the cooling jacket on the reactor chamber and connect them to each other, thereby bypassing the cooling jacket on the reactor chamber. Turn the water chiller ON and begin cooling the transducer.
- Set the ultrasonic amplitude to 80% (see BSP-1200 User Manual for details). Initiate ultrasound. Continue until the analog thermometer in the storage tank reaches 40–45 °C (104–113 °F) then stop the ultrasound and continue to recirculate the distilled water.



2 Adding mushroom extract, NanoStabilizer®-LSTM, and remaining water:

- a. Place the 7.5 L (2 gal) bucket on the digital scale and tare. Dispense **250 g** of your mushroom extract, **1 kg** of NanoStabilizer®-LSTM and **2 L** of distilled water into the bucket. Using the immersion blender, mix the mushroom extract, NanoStabilizer®-LSTM, and distilled water. Continue mixing until the pre-mix is homogenous, as shown below:



- b. Add the mushroom extract, Nanostabilizer®-LSTM, and distilled water pre-mix into the storage tank. Use the silicone spatula to ensure that the entire pre-mix is added to the storage tank.
- c. Repeat steps **2a** and **2b** three additional times so that the entire **1 kg** of mushroom extract, **4 kg** of Nanostabilizer®-LSTM, and **8 L** of distilled water have been pre-mixed and added to the storage tank.

3 Ultrasonic Processing:

In this step, ultrasonic processing will commence. Refer to BSP-1200 User Manual for operating instructions.

- a. Turn the water chiller OFF. Connect the cooling lines back to the cooling jacket on the reactor chamber, then turn the water chiller ON.

Note: Both the reactor chamber and transducer must be cooled during ultrasonic processing.
- b. Keep the ultrasonic amplitude at 80% (see BSP-1200 User Manual for details).

Note: The amplitude setting can be adjusted up or down to optimize the results.
- c. Activate ultrasound and start timing. Try to maintain the processed liquid temperature at 45–60 °C (113–140 °F) throughout processing (if the processed liquid becomes too cold, turn the water chiller OFF, temporarily disconnect the cooling lines from the cooling jacket on the reactor chamber and connect them to each other, then turn the water chiller ON to cool the transducer).
- d. **i) For users with access to particle size analysis (preferred).** After processing for 4 hours, draw a sample every 10 min and run the droplet size analysis. Once two consecutive samples demonstrate no significant decrease in the median droplet

size, deactivate ultrasound.

ii) For users without access to particle size analysis. Continue the process for about 4.5–5 hours. Deactivate ultrasound.

- e. Allow your nanoemulsion to stir, recirculate and cool for 15 min (the water chiller must be on and the reactor chamber must be cooled during this step). Once the nanoemulsion has cooled to 30–35 °C (86–95 °F), turn the water chiller OFF.
- f. Turn the pump OFF, reverse its direction and turn it ON again in order to collect all of the nanoemulsion from the tubing and the reactor chamber into the storage tank with mixer.
- g. Turn the pump OFF and return its direction to the original setting.

Filtration:

4

In this step, you will use the large-capacity in-line filter assembly with 1 micron cartridge to remove any particulate contamination from your nanoemulsion as you collect it in the finished product container.

Click the link or scan QR code below to see instructional video on filtration:

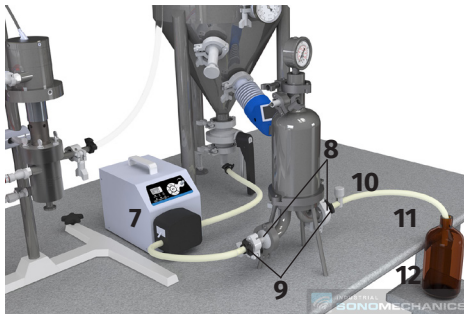
<https://youtu.be/NNSmQ3rN3As?si=5wwFZajECtA-t0e4>



PARTS NEEDED:



1. 1 micron filter cartridge
2. a. Filter housing top
b. Filter housing base
c. Bleed valve
3. 2x 1.5" sanitary clamp
4. 2x 1.5" sanitary gasket
5. 4" sanitary clamp
6. 4" sanitary gasket
7. Peristaltic pump with #36 silicone tubing
8. 2x 1.5" sanitary to 1/2" hose barb adapters
9. 2x plastic snap clip for #36 silicone tubing
10. Outlet pinch-valve
11. #36 silicone tubing for the filter outlet
12. Finished product container(s)



- a. Close the bottom outlet valve of the storage tank with mixer. Detach the outlet line from the reactor chamber and connect it to the inlet of the filter housing base (2b). Assemble the rest of the items 1 – 12 as shown above.
- b. Close the outlet pinch valve (10) by turning it clockwise. Open the bleed valve (2c) and open the bottom outlet valve of the storage tank with mixer.
- c. Set the pump (7) flow rate to 0.5 L/min (approximately 100 RPM), hold the 100 ml beaker underneath the bleed valve (2c), and be ready to turn the pump (7) OFF once the bleed valve (2c) starts to release the nanoemulsion. Turn the pump (7) ON and wait until the nanoemulsion starts to come out of the bleed valve (2c) then immediately turn the pump (7) OFF and close the bleed valve (2c). Add the nanoemulsion in the 100 ml beaker back into the storage tank. The filter housing top (2a) is now filled with your nanoemulsion. Open the pinch valve (10) by turning it counterclockwise.
- d. Turn the pump (7) ON and pass the nanoemulsion through the large-capacity in-line filter assembly into the first preseterilized finished product container (12). It is recommended to place the finished product container (12) below the level of the filter assembly. Once the presterilized finished product container is almost full, stop the pump and wait until the nanoemulsion stops flowing.

Note: The pressure gauge will show lower pressure at the start of the filtration

process, which is normal. A slight increase in pressure is expected as you filter more of the nanoemulsion. However, if the pressure reading rises significantly during filtration, it is likely time to clean or replace the filter cartridge.

- e.** Carefully remove the silicone tubing (**11**) from the filled presterilized finished product container (**12**) and place it into an empty presterilized finished product container (**12**). Tightly close the filled finished product container.
- f.** Repeat steps **d** and **e** until the entire 20 L of nanoemulsion has been filtered.
- g.** Store the finished product containers with the filtered nanoemulsion in a cool and dark place.
- h.** Gently flush the large-capacity in-line filter assembly by pumping distilled water through it in both directions until the water runs clean. Disassemble the assembly, remove the 1 micron filter cartridge and air-dry it on a dish rack.

To re-order NanoStabilizer®-LSTM and replacement filter cartridges, please use the link or scan QR code below to visit our online store.

<https://sonomechanics.myshopify.com>





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