**General protocol for customized inoculation of microbial culture within a small bioreactor platform (SBP)**

Instruments:

SBP capsules (Bio Castle)

Erlenmeyer 500ml

Beaker 250ml

Shaker incubator

Sterile Syringes 1 or 2.5ml

Sterile Needles 21G

Stand for placing capsules

Sterile absorbing paper/pad

Forceps

Reagents:

Mineral Salts Medium/M9 (MSM) (for procedure A)

Microorganisms' suitable growth medium (sterile, inside the 500ml Erlenmeyer)

Microorganism suspension in growth medium

Sealing polymer (liquid Cellulose Acetate) provided in a vial within the kit

Saline

Important Notes

(1) The inner membrane of the capsules is made of a water-soluble polymer (gelatin). Thus, in order to inoculate yeasts, molds, or any other non-producing gelatinase microorganism, it is highly recommended to pre-inoculate the SBP capsules, to degrade the gelatin internal component, prior to introducing the microorganism culture into the SBP capsules. This action will increase the possibility of the culture to adapt and proliferate within the capsule.

(2) Please work in a biological hood or other sterile environment.

**Procedure # A (Optional): Pre-inoculation of SBP capsules (gelatin removal)**

1. Immerse SBP capsules in MSM pH 2-3
2. Incubate at 50°C for 48hr in a shaker incubator with an orbital agitation of 100 rpm

Note: the medium might appear pink or radish at the end of the incubation period.

3. Transfer the SBP capsules into fresh MSM pH-7
4. Incubate for 3-4hr in a shaker incubator at 100rpm (Room Temperature)
5. Remove the capsules from the incubation solution and put them on a sterile absorbing paper/pad for 15 minutes to drain the water from the capsules.
6. The capsules are ready for inoculation.

**Procedure # B: Microorganism inoculation in SBP capsules**

1. Prepare microorganism suspension in appropriate growth medium at a desired concentration.
2. Arrange the capsules in an appropriate stand.
3. Draw the microorganism suspension using a 21G needle and 1-2.5ml syringe (transfer any excess into a waste beaker in the hood).
4. Insert 1ml of microorganism suspension into the capsule by piercing the capsule at one end using a syringe and 21G needle.
5. If more than one capsule is being used, then wipe the needle between fillings with a sterile absorbing paper/pad to prevent contamination outside the capsule.
6. Seal capsules with 5µl of sealing polymer, included in the kit, apply a drop of sealant onto the puncture hole.
7. Dry the sealing polymer in a biological hood for 5 minutes (or until seal appears white).
8. Repeat sealing stages (steps 6 - 7) X2
9. Activate capsules by incubating in saline with nutrients (LB 2%) (or any other appropriate medium) for 48hr (adjust the temperature and other conditions to the inserted culture)
10. Capsules are ready for use.

Troubleshoot:

| Problem | reason | action |
|---|---|---|
| **Medium Contamination** | Inserting a volume higher than 1 ml of microorganism suspension may cause a spill on capsule surface and contaminate the capsule outer membrane and medium. | (1) Prepare a new incubation medium and system.<br>(2) Transfer the capsules into ethanol 70% solution for 5 seconds.<br>(3) transfer the capsules to the new incubation system. |
| **Medium Contamination** | may occur due to a fracture, or nick, in the capsule membrane. | (1) Discard contaminated capsules.<br>(2) Prepare a new incubation medium and system.<br>(3) Transfer the capsules into ethanol 70% solution for 5 seconds.<br>(4) transfer the capsules to the new incubation system. |
| **Medium appears milky** | The capsule membrane is composed of Cellulose Acetate which releases fragments into the surrounding medium. | Replace Medium.<br>Do not air dry the capsule, do not leave capsules out of the medium for more than 10 seconds. |
| **Leakage through puncture hole** | Punctured filling hole is not sealed | (1) Discharge the unsealed capsules.<br>(2) Prepare a new incubation medium and system.<br>(3) Transfer the capsules into ethanol 70% solution for 5 seconds.<br>(4) transfer the capsules to the new incubation system. |
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FAQs:

1. Q: What material are the SBP capsules made of?

   A: The SBP capsules are composed of a microfiltration Cellulose Acetate membrane. The microfiltration membrane consists of 0.2 – 0.75µM pore sizes.

2. Q: Can Microorganisms pass through the membrane?

   A: The Capsules are intended for creating a physical barrier between the inner space and the outer surroundings with 0.2 – 0.75µM microfiltration. Therefore, no microorganism can traffic through the membrane, only dissolved nutrients and proteins.

3. Q: What kind of microorganisms can be inoculated inside the SBP capsules?

   A: It is possible to inoculate the capsule with bacteria, yeasts, and several species of molds. Working with yeast and other fungi cultures, it is recommended starting with procedure A prior to procedure B.

4. Q: For how long are the SBP capsules viable and functional?

   A: Depending on the type of microorganism inoculated in the capsule and other incubation terms (shear forces, temperature, pH etc.). The capsules may be used for up to eight weeks, it is recommended to use an additional number of capsules for monitoring viability and functionality.