KSB-3 Complete Medium® Kit

Description

The KSB-3 Complete Medium® Kit has been developed for culture of mesenchymal stem cells (MSCs).

Content of the Product

Product	Catalog no.	Size	Qty	Storage
KSB-3 Complete Medium® Kit	K3901	Kit	1	-
KSB-3 Basal Medium®	B1001	500mL	1	2-8℃*
KSB-3 Supplements®	S2901	2mL	1	-20℃*

* protect from light.

Important information

- KSB-3 Supplements® overnight at 4℃.

- KSB-3 Supplements® is stable up to at least one month at 2~8℃. Avoid refreeze.

- KSB-3 Complete Medium® is stable up to at least one month at 2~8℃.

Medium preparation

- Add KSB-3 Supplements® to KSB-3 Basal Medium® **(KSB-3 Complete Medium®).**

- KSB-3 Complete Medium requires supplementation with serum (commonly 5~10% Fetal Bovine Serum (FBS)).

- Antibiotic Supplement for Media (optional).

Guidelines for culture of mesenchymal stem cells (MSCs)

- We recommend refeeding the cultures every 2-3 days and passaging every 5-7 days.

- Do not let passaged MSCs become completely confluent, as it can reduce multipotency of MSCs.

- We recommend a seeding density of 3-6 x 10^3 viable cells/cm^2.

Thawing of Cryopreserved MSCs

1. Rapidly thaw frozen vial of cells in a 37℃ water bath.
2. Transfer the cells into a 50mL conical tube.
3. For every 1mL of cell suspension, add 10mL of KSB-3 Complete Medium®.
4. Centrifuge cells at 200-400 x g for 5 minutes.
5. Resuspend cells in KSB-3 Complete Medium®. Take an aliquot from the cell suspension for cell counting.
6. Seed cells into culture vessels.
7. Incubate at 37℃ in a humidified atmosphere containing 5% CO_2.
8. After 24hrs, discard spent medium and feed cells with fresh medium.
9. Refeed cells by complete medium exchanged every 2-3 days until cell passaging is needed.

Passing MSCs

1. Aspirate off spent medium.

2. Gently rinse cells with PBS and aspirate off.

3. Add the 0.05~0.25% Trypsin-EDTA. Incubate vessels at 37℃.

4. All the cells have detached, quickly proceed to the next step.

5. Add KSB-3 Complete Medium® to the vessel. Collect the cell suspension in a sterile 50mL conical tube.

6. Centrifuge cells at 200-400 x g for 5 minutes.

7. Remove supernatant.

8. Resuspend cells in KSB-3 Complete Medium®. Take an aliquot from the cell suspension for cell counting.

9. Seed cells into culture vessels.

10. Incubate at 37℃ in a humidified atmosphere containing 5% CO_2.

11. Refeed cells by complete medium exchanged every 2-3 days until cell passaging is needed.

Cryopreservation of cells

1. Prepare cryopreservation solution by supplementing the KSB-3 Complete Medium® with 10% dimethyl sulfoxide (DMSO).

2. Harvest cells as described in the passing protocol.

3. Pellet cells by centrifugation at 200-400 x g for 5 minutes, resuspend cells in the cryopreservation solution.

4. Take an aliquot from the cell suspension for cell counting.

5. Transfer to cryogenic vials. 1℃ freezing container and place in a -80℃ freezer overnight.

6. Transfer cryogenic vials to the liquid nitrogen for long-term storage.