

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	Shelf Life
KSB-3 Complete Medium® Kit	K3901	Kit	1	-	-
KSB-3 Basal Medium®	B1001	500mL	1	2-8°C*	12 months
KSB-3 Supplements®	S2901	2mL	1	-20°C*	12 months

* protect from light.

Important information

- KSB-3 Supplements® overnight at 4°C.
- KSB-3 Supplements® is stable up to at least one month at 2~8°C. Avoid refreeze.
- KSB-3 Complete Medium® is stable up to at least one month at 2~8°C.

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 $3-6 \times 10^3$ viable cells/cm².

Thawing of Cryopreserved MSCs

1. Rapidly thaw frozen vial of cells in a 37°C 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°C in a humidified atmosphere containing 5% CO₂.
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°C.
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°C in a humidified atmosphere containing 5% CO₂.
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°C freezing container and place in a -80°C freezer overnight.
6. Transfer cryogenic vials to the liquid nitrogen for long-term storage.

For Research Use Only. Not for use in diagnostic procedure.