L6565 Cell Line

Catalog No.: CL-0263

Origin and General Characteristics

Cell Name	L6565 Cell Line
Organism	Mus musculus, Mouse
Tissue	Peripheral blood
Morphology	Lymphoblast
Growth Properties	Suspension
Biosafety Level	1

Culture Conditions and Handling

Complete Growth Medium	RPMI-1640(PM150110)+10% FBS+1% P/S(PB180120)
Subculturing	Cultures can be maintained by the addition of fresh medium or replacement of medium. Alternatively, cultures can be established by centrifugation with subsequent resuspension at the subcultivation ratio of 1:2-1:4.
Subcultivation Ratio	1:2 to 1:4
Medium Renewal	Every 2 to 3 days
Cryopreservation	Freeze medium: 60% Basal medium+30% FBS+10% DMSO Storage temperature: Liquid nitrogen vapor phase
Culture Conditions	Atmosphere: Air, 95%; CO2, 5% Temperature: 37℃

Recommendations for handling of cryopreserved cells

1. The cell is packaged by dry ice. When receiving the cell, please make sure that the vial is still frozen. If there is cell thawing in the tube, please take photo before experiment or storage.
2. If immediate culturing is not intended, the cryovial(s) must be stored in liquid nitrogen (-196°C) or at least at -80°C after arrival.

   If immediate culturing is intended, please follow these instructions:
3. Quickly thaw by rapid agitation in a 37℃ water bath within 45-90 seconds. The water bath should have clean water containing an antimicrobial agent. As soon as the sample has thawed, remove the cryovial from the water bath.
   From now on, all operations should be carried out under aseptic conditions.
4. Transfer the cryovial to a sterile flow cabinet and wipe with 70% alcohol. Carefully open the vial and transfer the cell suspension into a 15 ml centrifuge tube containing 9 ml of cell complete medium (room temperature or 37℃).
5. In order to reduce cell damage, add 1ml of cell complete medium into cryovial, slightly pipette, then use a pipettor to add 1 ml of suspension into the centrifuge tube. Resuspend the cells carefully. Centrifuge at 300×g for 3 min and discard the supernatant. The centrifugation step may be omitted, but in this case the remains of the freeze medium have to be removed 24 hours later.
6. Resuspend the cells carefully in 10ml fresh cell culture medium and transfer them into one or two T25 cell culture flasks.

Precautions after receipt of frozen cells

1. After unpacking, check the status of the cells and dry ice, and take photos at the same time (The following service after sales will be provided according to the photos): such as the amount of dry ice remaining, whether the frozen cells are hided in the dry ice, and whether the cells have thawed and frozen again.
2. The received cells should be transferred to liquid nitrogen immediately or directly resuscitated. If there is no liquid nitrogen, they can be temporarily stored in a -80℃ refrigerator, but the storage time should be less than two weeks as much as possible. Because the vitality of resuscitated cells will gradually decrease with the extension of storage time, and the rate of this vitality decline is unpredictable.
3. Ensure that the operator has the knowledge and experience in cell culture, and make sure that the laboratory has basic instruments or equipment (biosafety cabinet, CO2 incubator, inverted microscope, centrifuge, water bath, etc.), and carefully read the cell instructions to understand cell-related information, such as growth properties (adherent/suspension), morphology, basic medium, serum concentration, cytokines, subcultivation ratio, medium renewal, etc.
4. When resuscitating the cells, quickly thaw the cells by gently swirling the vial in the 37°C water bath (The cell cryovial can be placed into disposable PE gloves to avoid cell contamination caused by water infiltration from water bath into the vial), and try to melt the cells within 1 min to avoid the formation of internal ice crystals in the cells to cause the cells to break and die.
5. After the cells are resuscitated, the cells can be observed under a microscope and the cell state can be recorded by taking photos (take 1-3 pictures for each of 100× and 200×, for 3 consecutive days, the photos taken will be used as the basis for follow-up services). At the same time, a small amount of cells can be taken for the detection of cell viability (counting by cell counter or trypan blue staining). Note: Cells within 24 hours of resuscitation should not be observed frequently, otherwise it will affect cell growth or adherence, just 1-2 times a day is ok.
6. The cells should be cryopreserved as soon as possible after expanding the culture, in order to have the cells to do the same expeirment if there is the unexpected during the expirement. It is worth noting it should choose the cells in the logarithmic growth phase when cryopreserving cells. After cryopreservation, it needs to resuscite a vial of cells to test the vitality to confirm whether the cryopreserved is OK.
7. The time of refund/replacement: In principle, the cells can grow and passage normally after resuscitating, or over one month after the cell is received, the refund/replacement will no longer be provided.

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