

RAW 264.7 Cell Line

Catalog No.: CL-0190

Origin and General Characteristics

Cell Name	RAW 264.7 Cell Line
Organism	Mus musculus, Mouse
Age	Adult
Tissue	Abelson murine leukemia virus-induced tumor, ascites
Morphology	Monocyte/macrophage
Growth Properties	Adherent
Descriptions	This cell line is easy to propagate, high efficiency for DNA transfection, sensitivity to RNA interference, and supports replication of murine noroviruses. This cell line is negative for surface immunoglobulin(sIg-), Ia(Ia-) and Thy-1.2(Thy-1.2). When this line was established, it was described as not secreting detectable virus particles and negative using the XC plaque formation assay. Based on a published study by Dr. Janet W. Hartley in 2008, this line was demonstrated to express ecotropic and polytropic MuLV, and is positive using the XC plaque assay for virus replication.Ref
Biosafety Level	2

Culture Conditions and Handling

Complete Growth Medium	DMEM(PM150210)+10% FBS+1% P/S(PB180120)
Subculturing	Subcultures are prepared by scraping or blowing. For a 100mm disk,remove all but 10 mL culture medium (adjust amount accordingly for otherculture vessels). Dislodge cells from the flask substrate with a cell scraperor blowing directly with the pipettes; aspirate and add appropriatealiquots of the cell suspension into new culture vessels.
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°C

Special Features of the Cell Line

Applications	This cell line is a suitable transfection host. The cells will pinocytose neutral red and will phagocytose latex beads and zymosan. They are capable of antibody dependent lysis of sheep erythrocytes and tumor cell targets. LPS or PPD treatment for 2 days
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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°C 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°C).
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°C 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 experiment if there is the unexpected during the experiment. It is worth noting it should choose the cells in the logarithmic growth phase when cryopreserving cells. After cryopreservation, it needs to resuscitate 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.