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Cell culture of J774A.1 cells

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We use this protocol and it's working

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Abstract

The J774 tumor arose in a female BALB/c/NIH mouse with a reticulum cell sarcoma during a plasmacytoma induction program in 1968 [1]. The original tumor from which the cells designated J774A.1 were isolated was ascitic. J774A.1 macrophages are active in antibody-dependent phagocytosis [2]. Their growth is inhibited by dextran sulfate, p-phenylenediamine and LPS. J774A.1 cells synthesize large amounts of lysozyme and exhibit low cytolysis but predominantly antibody-dependent phagocytosis. Interleukin-1β is continuously synthesized by this cell line [3]. J774A.1 cells have a doubling time of 17 hours.

Materials

Equipment:

- Personal protective equipment (sterile gloves, laboratory coat, safety visor etc.)
- Water bath set to 37 °C
- Microbiological safety cabinet at the appropriate containment level
- Incubator at 37 °C and 5 % (v/v) CO_2 atmosphere
- Centrifuge
- Inverted microscope
- Neubauer counting chamber and cover slips or cell counter
- Freeze container (e.g. Mr. Frosty, Nalgene)
- - 80 °C freezer
- Liquid nitrogen tank

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Materials:

- Cell culture flasks: 25 and 150 cm^2
- Sterile serological pipette
- Sterile Pasteur pipette
- Sterile filter tips
- Sterile reaction tubes (15 and 50 ml)
- Sterile cell scraper
- Sterile cryotubes (2 ml)

Chemicals:

- Dulbecco's Modified Eagle's Medium (4500 mg/L glucose, sterile, suitable for cell culture) pre-warmed to 37 °C
- Fetal bovine serum (Merck/Sigma Aldrich, Cat. No. S0615)
- L-Glutamine–penicillin–streptomycin solution (L-glutamine 200 mM, streptomycin 10 mg/mL, penicillin 10,000 units)
- Sterile dimethyl sulfoxide (DMSO)
- 2-Propanol

Troubleshooting

Thaw J774A.1 cells

1 Transfer the cryotube with the cell suspension from the liquid nitrogen tank to the cell culture on ice.

Note: When handling with liquid nitrogen, wear protective goggles, gloves and a gown, otherwise cold burn may occur.

2 Thaw the cells immediately in a 37 °C water bath until no more ice chunks are visible.

Note: This procedure and the next steps must be performed quickly because the cryoprotectant dimethyl sulfoxide (DMSO) is cytotoxic above 4 °C.

3 Mix the thawed 1 ml cell suspension with 14 ml pre-warmed high-glucose Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10 % (v/v) fetal bovine serum (FBS) and 0.1 mg/ml glutamine–penicillin–streptomycin (PSG) solution in a 15 ml tube and resuspend well but gently.

4 Centrifuge the cell suspension for 10 min with 300 x g at room temperature and carefully aspirate the supernatant with a sterile pasteur pipette.

5 Resuspend the cell pellet in 5 ml fresh high-glucose DMEM (containing 10 % (v/v) FBS and 0.1 mg/ml PSG) and transfer the cell suspension to a 25 cm^2 cell culture flask and place it in a 37 °C incubator with 5 % (v/v) CO_2 atmosphere for growth.

6 Leave the cells in the incubator and observe the cell growth every two days. Once the cells reach 60 to 80 % confluency, remove the supernatant and detach the adherent cells in 5 ml fresh high-glucose DMEM (containing 10 % (v/v) FBS and 0.1 mg/ml PSG) using a cell scraper.

7 Fill the cell suspension with fresh pre-warmed high-glucose DMEM (containing 10 % (v/v) FBS and 0.1 mg/ml PSG) to a total volume of 50 ml and transfer the whole cell suspension into a 150 cm^2 cell culture flask and put the flask in a 37 °C incubator with a 5 % (v/v) CO_2 atmosphere.

Passaging J774A.1 cells

8 Control the cells using an inverted microscope to assess the degree of confluency, check for morphological changes, and confirm the absence of bacterial and fungal contamination.

Note: Elongated cells indicate activation, non-activated cells have a round shape.

9 For a 150 cm^2 cell culture flask, transfer the whole used DMEM in a tube (50 ml) and centrifuge at 400 x g for 5 min at room temperature.

Note: To avoid contamination, all steps onwards should be performed in a sterile environment.

10 Add 10 ml fresh high-glucose DMEM (containing 10 % (v/v) FBS and 0.1 mg/ml PSG) to the cell monolayer and aspirate the supernatant.

Note: High-glucose DMEM (containing 10 % (v/v) FBS and 0.1 mg/ml PSG) should be pre-warmed to 37 °C. During this procedure, the cells should be placed on a 37 °C pre-heated thermoplate.

11 Add an additional 10 ml fresh high-glucose DMEM (containing 10 % (v/v) FBS and 0.1 mg/ml PSG), detach cells carefully but with sufficient pressure with a cell scraper, and transfer the whole cell suspension into a new tube.

12 Label the cell culture flask with the date and the number of passaging.

Note: Under normal conditions, one cell culture flask can be used for 10 to 20 cell passages. If significantly more cells are observed in the supernatant (indicating dead cells) during the morphological evaluation, the cell culture flask should be replaced with a new one accordingly.

13 To passage the cells, the cell suspension is diluted (at 80 % confluency choose a 1:5 dilution with high-glucose DMEM (containing 10 % (v/v) FBS and 0.1 mg/ml PSG), referring to total volume of 10 ml.

14 Transfer the defined amount (e.g. 2 ml at a 1:5 dilution) back into the culture flask and add 48 ml fresh high-glucose DMEM (containing 10 % (v/v) FBS and 0.1 mg/ml PSG).

15 When subcultured cells reach 60 to 80 % confluency (normally 2 to 3 days), repeat the cell passaging as outlined above.

Seeding J774A.1 cells for subsequent experiments

16 Use the remaining cell suspension from the subculture for seeding cells for further experiments.

17 Use the Neubauer counting chamber (or an automated cell counter) to determine the cell number per milliliter cell suspension.

Note: If using a Neubauer counting chamber, a total of 10 µl cell suspension should be pipetted under the cover slip. Usually, a 1:20 dilution of the suspension of J774A.1 cells is

needed to count the cell number using a microscope.

18 Prepare the required volume for seeding the cell suspension with fresh high-glucose DMEM (containing 0.1 mg/ml PSG).

One example for the calculation:

	A	B
	Determined cell number of suspension	4 × 10^6 cells per ml
	Total volume for seeding	5 ml
	Required cell number for seeding	3 × 10^6 cells in 5 ml
	Calculation:	
	Required cell suspension	0.75 ml
	Fresh high-glucose DMEM (containing 0.1 mg/ml PSG) to add	4.25 ml

19 Transfer the prepared cell suspension into culture plates or cell culture flasks as required.

Note: Depending on the experiment (e.g. with regard to the duration of incubation) and the research question, it may be useful or even necessary to adjust the cell density.

	A	B	C	D	E	F	G
		25 cm2-flask	6-well plate	12-well plate	24-well plate	48-well plate	96-well plate
	Cell number/flask or well	3 × 10^6	1 × 10^6	0.5 x 10^6	0.25 x 10^6	0.1 × 10^6	0.05 x 10^6
	Volume/flask or well	5 ml	2 ml	1 ml	0.5 ml	0.3 ml	0.2 ml

20 Label the cell culture vessel with the date and passage number of the cell culture. Put the seeded cells back into the 37 °C incubator with a 5 % (v/v) CO_2 atmosphere and allow the cells to settle for 24 h.

Freeze J774A.1 cells

21 Prepare freeze container: Fill the freeze container (e.g. Mr. Frosty, Nalgene) with 2-propanol and cool it at 4 °C. Cooling in 2-propanol ensures a slow and gentle temperature decrease. A cooling rate of -1°C/min is the optimal rate for cell preservation.

22 Prepare freezing medium: high-glucose DMEM containing 20 % (v/v) FBS, 0.1 mg/ml PSG and 10 % (v/v) sterile DMSO.

23 Count the cells with a Neubauer counting chamber or a cell counter, calculate the number of needed cryotubes and freezing medium (see the section "Seeding J774A.1 cells for subsequent experiments").
Adjust cell concentration to 1×10^7 cells/ml.

24 **Note:** Cells should be kept on ice from now on.

Distribute 1 ml of the cell suspension in freezing medium into each cryotube. Place the cryotube into the freeze container and subsequently put the freeze container into a - 80 °C freezer for cooling down overnight. On the next day, place the frozen cryotubes into liquid nitrogen for long term storage.

Protocol references

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