Sigma-Aldrich®

Data Sheet

B16-OVA MO4 Mouse Melanoma Cell Line

Cancer Cell Line

Cat. # SCC420

Pack size: ≥1x10^6 viable cells/vial

FOR RESEARCH USE ONLY
NOT FOR USE IN DIAGNOSTIC PROCEDURES
NOT FOR HUMAN OR ANIMAL CONSUMPTION

Store in liquid nitrogen

Background

The immune system can recognize and destroy tumors. Cytotoxic T lymphocytes (CTLs) kill neoplastic or virally infected cells after recognizing antigenic peptides bound to major histocompatibility complex class I molecules on their surface. These peptides are derived from antigens that are degraded in the cytosol of the affected cell. Immunizations with killed pathogens or their proteins do not generally elicit CTLs, because exogenous proteins cannot enter the cytosol (to be presented). However, antigens that are internalized into phagocytic cells can enter the cytosol and be processed for class I presentation. Immunization with a purified antigen attached to an avidly phagocytized particle primes CTLs, which in turn protect animals from subsequent challenge with tumors transfected with the antigen gene.[1]

Source

Tumor rejection antigens – TRAs – differ from any other protein synthesized by the cell in that the host is intolerant to them. Thus virtually any foreign protein synthesized by a tumor, including those expressed from foreign antigen genes transfected into tumor cells, should function and behave like TRAs. This hypothesis was tested in a murine tumor model developed by transfecting the chicken ovalbumin gene into the C57BL/6 (H-2b haplotype)-derived murine melanoma cell line B16[2], followed by selection and isolation of the ovalbumin (OVA)-transfected B16 clone B16-OVA MO4.[1]

In *in vitro* challenge, B16-OVA MO4 cells stimulated the OVA+ Kb-specific T-cell hybridoma RF33.70 to produce interleukin-2 whereas untransfected B16 cells did not. Injected intradermally into syngeneic C57Bl/6 mice, both B16 and B16-OVA MO4 grew progressively, metastasized, and killed the animals showing that expression of the ovalbumin antigen alone does not render this tumor sufficiently immunogenic to be rejected. Subcutaneous immunization of C57Bl/6 mice with ovalbumin conjugated to iron beads (OVA-Fe), however, protected B16-OVA MO4 (but not B16) challenged animals both from local tumor growth and death. This approach could be exploited to develop tumor and viral vaccines.[1]

Note: Mouse xenotropic retrovirus Bxv-1 proviral DNA is detected in B16-OVA MO4 cells, BXV-1 is a Biosafety Level 2 (BSL-2) pathogen.

Short Tandem Repeat (STR profile)

M1-1: 17, 18	M5-5: 16, 20	M11-2: 16, 17	M18-3: 15, 16
M1-2: 19, 20	M6-4: 18, 19	M12-1: 17, 18	M19-2: 13
M2-1: 16, 17	M6-7: 15, 16	M13-1: 17, 18	MX-1: 28
M3-2: 14, 15	M7-1: 26.2	M15-3: 22.3, 23.3	
M4-2: 20.3, 21.3	M8-1: 16, 17	M17-2: 15, 16, 17	

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Quality Control Testing

- Each vial contains ≥ 1X10^6 viable cells.
- B16-OVA MO4 cells are verified to be of mouse origin and negative for rat, Chinese hamster, Golden Syrian hamster, human, and non-human primate interspecies contamination, as assessed by a Contamination Clear panel by Charles River Animal Diagnostic Services.
- Cells tested negative for infectious diseases against the Mouse Essential CLEAR panel by Charles River Animal Diagnostic Services.
- Cells tested negative for mycoplasma.

Storage and Handling

B16-OVA MO4 cells should be stored in liquid nitrogen until use. The cells can be cultured for at least 10 passages after initial thawing without significantly affecting cell marker expression and functionality.

Representative Data

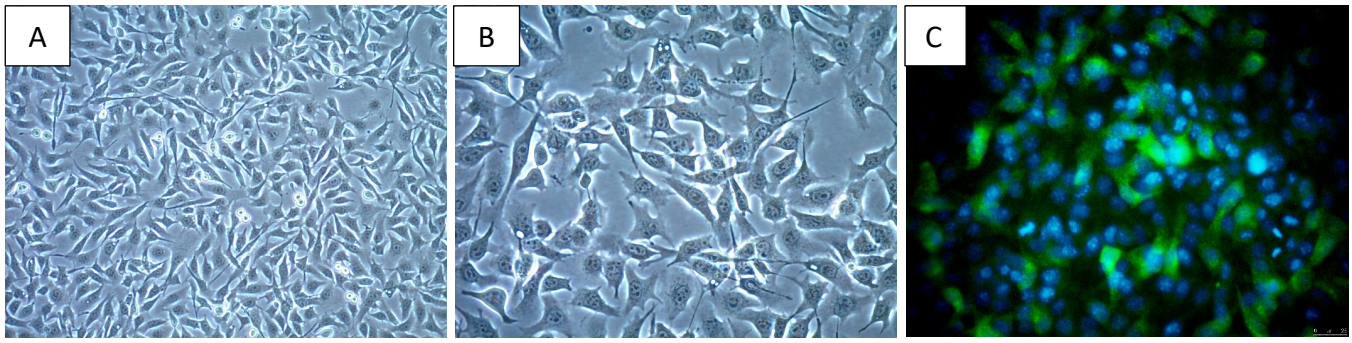

Figure 1. Bright-field image of B16-OVA MO4 at lower (A) and higher magnification (B). B16-OVA MO4 cells express ovalbumin (ABS818, C).

Protocols

Thawing the Cells

Do not thaw the cells until the recommended medium and coated cultureware are on hand.

1. Cells are expanded in B16-OVA MO4 Expansion Medium comprising RPMI-1640 (Sigma R8758), supplemented with 10% FBS (Sigma ES-009-B), 1X Non-Essential Amino Acids (Sigma TMS-001-C), 10 mM HEPES (Sigma TMS-003-C), 54 μM β-mercaptoethanol (Sigma ES-007-E) and 1 mg/ml Geneticin (G418; Sigma 345810).

   Note: For thawing, omit G418 from the Expansion Medium (Thawing Medium).
2. Remove the vial of frozen B16-OVA MO4 cells from liquid nitrogen and incubate in a 37°C water bath. Closely monitor until the cells are completely thawed. Maximum cell viability is dependent on the rapid and complete thawing of frozen cells. **IMPORTANT: Do not vortex the cells.**
3. As soon as the cells are completely thawed, disinfect the outside of the vial with 70% ethanol. Proceed immediately to the next step.
4. In a laminar flow hood, use a 1 or 2 mL pipette to transfer the cells to a sterile 15 mL conical tube. Be careful not to introduce any bubbles during the transfer process.

5. Using a 10 mL pipette, slowly add dropwise 9 mL of Thawing Medium (Step 1 above) to the 15 mL conical tube. **IMPORTANT: Do not add the entire volume of media all at once to the cells. This may result in decreased cell viability due to osmotic shock.**
6. Gently mix the cell suspension by slowly pipetting up and down twice. Be careful not to introduce any bubbles.
   IMPORTANT: Do not vortex the cells.
7. Centrifuge the tube at 300 x g for 2-3 minutes to pellet the cells.
8. Decant as much of the supernatant as possible. Steps 5-8 are necessary to remove residual cryopreservative (DMSO).
9. Resuspend the cells in 15 mL of Thawing Medium.
10. Transfer the cell mixture to a T75 tissue culture flask.
11. Incubate the cells at 37°C in a humidified incubator with 5% CO_2.
12. The next day, replace the Thawing Medium with B16-OVA MO4 Expansion Medium

Subculturing the Cells

Note: B16-OVA MO4 cells proliferate very rapidly with a population doubling time under 24 hours.

1. B16-OVA MO4 cells are adherent and to grow in monolayer. B16-OVA MO4 cells should be passaged at ~ 80% confluency.
2. Carefully remove the medium from the T75 tissue culture flask containing the 80% confluent layer of B16-OVA MO4.
3. Rinse the flask with 10 mL 1X PBS. Aspirate after the rinse.
4. Apply 5-7 mL of Accutase and incubate in a 37°C incubator for 3-5 minutes.
5. Inspect the flask and ensure the complete detachment of cells by gently tapping the side of the flask with the palm of your hand.
6. Add 5-7 mL of B16-OVA MO4 Expansion Medium to the plate.
   Note: Expansion Medium should containg 1 mg/mL G418.
7. Gently rotate the flask to mix the cell suspension. Transfer the dissociated cells to a 15 mL conical tube.
8. Centrifuge the tube at 300 x g for 3-5 minutes to pellet the cells.
9. Discard the supernatant, then loosen the cell pellet by tapping the tip of the tube with a finger.
10. Apply 2-5 mL of B16-OVA MO4 Expansion Medium to the conical tube and resuspend the cells thoroughly. Cell clumps may be broken up by extensive tituration using a 5 ml serological pipette.
    IMPORTANT: Do not vortex the cells.
11. Count the number of cells using a hemocytometer.
12. Plate the cells to the desired density. Typical split ratio is 1:6. **Note:** Cells proliferate rapidly and thus it is recommended to thaw the cells into a T175 flask.

Cryopreservation of the Cells

B16-OVA MO4 Mouse Melanoma Cells may be frozen in B16-OVA MO4 Thawing Medium supplemented with 10% DMSO using a Nalgene slow freeze Mr. Frosty container.

References

1. Falo LD Jr, Kovacsovics-Bankowski M, Thompson K, Rock KL. *Nat. Med.* 1995; 1(7):649-653.
2. Fidler IJ. *Cancer Res.* 1975; 35(1):218-224.

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