Product Information Sheet for NR-51842

Murine Cas9 Positive Conditionally-Immortalized Macrophage Progenitor Cell Line

Catalog No. NR-51842

For research use only. Not for use in humans.

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Manufacturer:
BEI Resources

Product Description:
Conditionally-immortalized macrophage progenitor cell line is derived from Cas9$^+$ murine hematopoietic stem cells ectopically expressing the estrogen-regulated version of homeobox transcription factor Hoxb8 (ER-Hoxb8). Presence of β-estradiol in the culture medium enables the self-renewal of macrophage progenitors, and its removal leads to differentiation of these to functional macrophage cell type. The estrogen-regulated Cas9-expressing (Cas9$^+$) macrophage progenitor cell line is an effective scalable system to utilize CRISPR/Cas9 technology to generate genetically modified macrophage populations. NR-51842 is grown in suspension, in specialized media described in the Thawing and Growth section of this document.[1,2]

Material Provided:
Each vial contains approximately 1 mL of cell culture suspension frozen in fetal bovine serum (FBS) (90%) and 5% dimethylsulfoxide (DMSO) (10%) cryopreservative. Sufficient cells are provided to initiate at least one new culture. The cell count, expressed as cells per vial, is shown on individual Certificates of Analysis for each lot.

Packaging/Storage:
NR-51842 was packaged aseptically, in screw-capped plastic cryovials. The product should be stored at -100°C or colder, preferably in the vapor phase of a liquid nitrogen freezer. Storage at -70°C will result in loss of viability.

Note: Do not under any circumstances store vials at temperatures warmer than -70°C. Storage under these conditions will result in the death of the culture.

To ensure the highest level of viability, the vial should be thawed, and the culture initiated as soon as possible upon receipt. Any warming of the product during shipping and transfer must be avoided, as this will adversely affect the viability of the product after thawing. For transfer between freezers and shipping, the cells may be placed on dry ice for brief periods, although use of a portable liquid nitrogen carrier is preferred. Please read the following recommendations prior to reconstituting this material.

Safety Precautions:
When handling frozen vials, it is highly recommended that protective gloves, lab coat and full-face mask be worn. Even brief exposure to the ultra-cold temperature can cause tissue damage from frostbite. Also, some vials may slowly fill with liquid nitrogen if they have been immersed during cryogenic storage. When thawing, the liquid nitrogen may rapidly expand as it changes to gas, breaking the vial or cap with explosive force, sending debris flying with enough velocity to cause injury. Store and use in areas with adequate ventilation.

Thawing and Growth:
Prior to thawing the murine macrophage cells, prepare growth medium (GM) for use (Appendix I). This GM is formulated for use in an aerobic atmosphere with 5% CO_2.

Rapidly thaw the vial of murine macrophage cells in a 37°C water bath. To reduce the risk of contamination, keep the cap and O-ring of the vial out of the water and repeatedly check the cap for tightness during thawing. Remove from the water bath immediately when thawed. Dry the vial with a sterile wiper, decontaminate using a wiper soaked with 70% isopropyl alcohol and let the vial air dry. Aseptically open the vial, remove the vial contents and add to 4 mL of GM in a centrifuge tube. Centrifuge the cell suspension at 125 × g for 8 to 10 minutes at 18 to 25°C. Discard the supernatant and resuspend the cell pellet in 10 mL of pre-warmed GM. Transfer the cell suspension into a 75 cm^2 tissue culture flask. Incubate the new culture at 37°C in an aerobic atmosphere with 5% CO_2.

Sub-culture procedure:
Aseptically transfer the contents of the flask to a centrifuge tube containing an equal volume of GM. Centrifuge the cell suspension at 125 × g for 8 to 10 minutes at 18 to 25°C. Discard the supernatant and resuspend the cell pellet in an equal amount of pre-warmed GM as was started with. Perform a cell count and add appropriate aliquots of the cell suspension to as many 75 cm^2 tissue culture flasks as needed to yield a sub-cultivation ratio of 1:3 to 1:4. Adjust the volume of GM to 15 to 20 mL for a 75 cm^2 flask. Incubate the new culture at 37°C in an aerobic atmosphere with 5% CO_2.

Citation:
Acknowledgment for publications should read "The following reagent was obtained through BEI Resources, NIAID, NIH: Murine Cas9 Positive Conditionally-Immortalized Macrophage Progenitor Cell Line, NR-51842."

Biosafety Level: 1
Appropriate safety procedures should always be used with this material. Laboratory safety is discussed in the following publication: U.S. Department of Health and Human Services, Public Health Service, Centers for Disease Control and Prevention, and National Institutes of Health. Biosafety in Microbiological and Biomedical Laboratories. 6th ed. Washington, DC: U.S. Government Printing Office, 2020; see www.cdc.gov/biosafety/publications/bmbl5/index.htm.

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References:

1. Cox, J., Personal Communication.
2. Roberts, A. W., et al. "Cas9+ Conditionally-Immortalized Macrophages as a Tool for Bacterial Pathogenesis and Beyond." eLIFE 8 (2019). doi: 10.7554/eLife.45957. PubMed: 31204998.

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APPENDIX I: Cas9$^+$ GROWTH MEDIUM

1. Prepare the bulk Cas9$^+$ incomplete growth medium by supplementing RPMI-1640 medium with the following components, and use within 30 days from preparation or discard[1]:

RPMI-1640 medium[2,3]	5.0 g
L-glutamine	2mM
HEPES [4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid]	10mM
β-estradiol	2 µM
Fetal bovine serum	10%

[1]Prepare sterile stock solutions at concentrations that are easily diluted into the liquid medium to obtain the appropriate user concentrations and add aseptically. Ready-made stock solutions for many of the components are available from numerous manufacturers.
[2]RPMI-1640 medium is available from numerous manufacturers as both a powder and a sterile, prepared liquid, with or without L-glutamine and HEPES. If using powdered RPMI-1640 medium, prepare the medium following manufacturer instructions, sterile-filter using a 0.22 µm filter, then aseptically add the necessary components in the appropriate concentrations.
[3]If stock solutions were not sterile or aseptic techniques were not followed, sterile-filter the medium using a 0.22 µm filter after the addition of all components. Store at 4°C.

2. Prepare the working medium by supplementing the bulk Cas9$^+$ incomplete growth medium with the following reagents directly before use. These supplements are only stable in media for up to 7 days and must be spiked fresh into an aliquot of bulk media at the time of fluid addition or fluid change:

β-mercaptoethanol	43 µM
B16 murine melanoma cell line GM-CSF	2 ng per mL

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