Cell Line Designation: L-Wnt-3A

AddexBio Catalog No. S0011001

Cell Line Description:

Applications: This cell line is currently the best source for producing Wnt-3A conditioned medium. This cell line was derived from L-M(TK-) cells that were transfected with a Wnt-3A expression vector and stable clones were selected in medium containing G418.

Species: Mus musculus, mouse

Tissue: Subcutaneous connective tissue; areolar and adipose

Properties: Adherent; fibroblasts

Complete Medium: AddexBio-formulated DMEM Medium (C0003-01) + G-418 (0.4 mg/mL) + 10% FBS

Subculture Procedure: A subcultivation ratio of 1:3 to 1:10 using 0.25% trypsin or trypsin/EDTA, 5% CO2; 37°C. Please apply antibiotic one day after splitting the cells to avoid killing of the cells before they have a chance to attach to the flask.

Protocol for Wnt-3A Conditioned Medium:

Split the cells 1:10 in culture medium (without G418 if conditioned medium is to be used with a cell line sensitive to G418) in 10 cm tissue culture dishes or T75 flasks and let the cells grow for 4 days (approximagely to confluency).

Take off the medium and sterile filter. This is the first batch of medium.

Add 10 mL fresh culture medium and culture for another 3 days.

Take off the medium and sterile filter. This is the second batch of medium. Discard the cells, because they will be overgrown.

Mix the first batch and second batch of medium 1:1. This is the Wnt-3A conditioned medium. It is stable at 4°C and can be frozen.

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Medium Renewal: Two to three times weekly.

Freezing Medium: Complete culture medium supplemented with 5% (v/v) DMSO

Additional Information: Additional product and technical information can be obtained from the catalog references and the Addexbio Technical Information site at www.addexbio.com, or by email at customersupport@addexbio.com.

Biosafety Level: 1

Appropriate safety procedures should always be used with this material. laboratory safety is discussed in the following publication: Biosafety in Microbiological and Biomedical Laboratories, 5th ed. HHS Publication No. (CDC) 93-8395. U.S. Department of Health and Human Services, Centers for Disease Control and Prevention. Washington DC: U.S. Government Printing Office; 2007. The entire text is also available online at www.cdc.gov/od/ohs/biosafty/bmbl4/bmbl4toc.htm

Use Restrictions: **These cells are distributed for research purposes only.** Addexbio does not recommend third party distribution of this cell line, as this practice has resulted in the unintentional spreading of contaminated cell lines.

Handling Procedure for Frozen Cells:

To insure the highest level of viability, thaw the vial and initiate the culture as soon as possible upon receipt. If upon arrival, continued storage of the frozen culture is necessary, it should be stored in liquid nitrogen vapor phase and not at -70°C. Storage at -70°C will result in loss of viability.

Safety Precaution:

Addexbio highly recommends that protective gloves and clothing always be used and a full face mask always be worn when handling frozen vials. It is important to note that some vials leak when submersed in liquid nitrogen and will slowly fill with liquid nitrogen. Upon thawing, the conversion of the liquid nitrogen back to its gas phase may result in the vessel exploding or blowing off its cap with dangerous force creating flying debris.

1. Thaw the vial by gentle agitation in a 37°C water bath. To reduce the possibility of contamination, keep the O-ring and cap out of water. Thawing should be rapid (approximately 2 minutes).

2. Remove the vial from the water bath as soon as the contents are thawed, and decontaminate by dipping in or spraying with 70% ethanol. All of the operations from this point on should be carried out under strict aseptic conditions.

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3. Resuspend cell pellet with the recommended complete medium and dispense into a new culture flask. It is important to avoid excessive alkalinity of the medium during recovery of the cells. It is suggested that, prior to the addition of the vial contents, the culture vessel containing the complete growth medium be placed into the incubator for at least 15 minutes to allow the medium to reach its normal pH (7.0-7.6).

4. Incubate the culture at 37°C in a suitable incubator. A 5% CO_2 in air atmosphere is recommended.

References for L-Wnt-3A cells:

1. Willert K, Brown JD, Danenberg E, Duncan AW, Weissman IL, Reya T, Yates JR 3rd, Nusse R. Wnt proteins are lipid-modified and can act as stem cell growth factors. Nature. 2003 May 22;423(6938):448-452.

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Lot Specific Information Sheet for AddexBio Cat #: S0011001

Lot Number: 0328938

Designation: L-Wnt-3A CELLS

Total Cells/mL: >$1.6x10^6$

Expected Viability: >76.5%

Ampule Passage #: 10

Dilute Ampule Content: 1:10 (T-25) or 1:15 (T-75)

Volume/Ampule: 1 mL

A T-75 setup at a seeding density of 1.5×10^4 viable cells/cm^2 reaches approximately 20% confluence in 2 days.

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