Product sheet

L-WRN Cells | 300641

General information

Description	The L-WRN cell line is a murine fibroblast cell line derived from the L cells, which are mouse fibroblasts originally isolated from connective tissue. L-WRN cells have been engineered to stably express Wnt3a, R-spondin 3, and Noggin. These factors are critical for the growth and maintenance of intestinal organoids and stem cell cultures. The overexpression of these proteins enhances the proliferation and differentiation of intestinal stem cells, making L-WRN cells a valuable tool for studying intestinal biology and disease modeling. In addition to their application in organoid culture, L-WRN cells serve as a robust model for investigating Wnt signaling pathways. Wnt signaling is pivotal in regulating cell fate, proliferation, and migration during development and in adult tissues. By providing a consistent and controlled source of Wnt3a, R-spondin 3, and Noggin, L-WRN cells facilitate research into the molecular mechanisms underlying these processes. Researchers can use these cells to dissect the roles of these signaling molecules in various biological contexts, including cancer, tissue regeneration, and developmental biology. Overall, the L-WRN cell line is a powerful tool in biomedical research due to its ability to support the growth of complex three-dimensional cultures and its utility in studying key signaling pathways. Its role in the advancement of intestinal stem cell research and its contributions to our understanding of Wnt signaling highlight its importance in the field of cellular and molecular biology.
Organism	Mouse
Tissue	Connective tissue
Applications	3D cell culture

Characteristics

Breed/Subspecies	C3H/An
Age	100 days
Gender	Male
Morphology	Fibroblast
Growth properties	Adherent

Regulatory Data

Citation	L-WRN (Cytion catalog number 300641)

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Biosafety level	1
NCBI_TaxID	9606
CellosaurusAccession	CVCL_DA06
GMO Status	GMO-S1: This murine NIH-3T3-derived cell line (L-WRN) contains expression constructs for Wnt3a, R-spondin-3, and Noggin, including SV40 DNA sequences and dual antibiotic markers (hph and Tn5-neo), enabling secretion of these signaling molecules. The inserts are stably present in NIH-3T3-based cells. This classification applies only within Germany and may differ elsewhere.

Biomolecular Data

Protein expression	Wnt-3A, R-spondin, noggin

Handling

Culture Medium	DMEM, w: 4.5 g/L Glucose, w: 4 mM L-Glutamine, w: 3.7 g/L NaHCO3, w: 1.0 mM Sodium pyruvate (Cytion article number 820300a)
Supplements	Supplement the medium with 10% FBS
Dissociation Reagent	Accutase
Subculturing	Remove the old medium from the adherent cells and wash them with PBS that lacks calcium and magnesium. For T25 flasks, use 3-5 ml of PBS, and for T75 flasks, use 5-10 ml. Then, cover the cells completely with Accutase, using 1-2 ml for T25 flasks and 2.5 ml for T75 flasks. Let the cells incubate at room temperature for 8-10 minutes to detach them. After incubation, gently mix the cells with 10 ml of medium to resuspend them, then centrifuge at 300xg for 3 minutes. Discard the supernatant, resuspend the cells in fresh medium, and transfer them into new flasks that already contain fresh medium.
Freeze medium	As a cryopreservation medium, we use complete growth medium (including FBS) + 10% DMSO for adequate post-thaw viability, or CM-1 (Cytion catalog number 800100), which includes optimized osmoprotectants and metabolic stabilizers to enhance recovery and reduce cryo-induced stress.

Thawing and Culturing Cells	1. Confirm that the vial remains deeply frozen upon delivery, as cells are shipped on dry ice to maintain optimal temperatures during transit. 2. Upon receipt, either store the cryovial immediately at temperatures below -150°C to ensure the preservation of cellular integrity, or proceed to step 3 if immediate culturing is required. 3. For immediate culturing, swiftly thaw the vial by immersing it in a 37°C water bath with clean water and an antimicrobial agent, agitating gently for 40-60 seconds until a small ice clump remains. 4. Perform all subsequent steps under sterile conditions in a flow hood, disinfecting the cryovial with 70% ethanol before opening. 5. Carefully open the disinfected vial and transfer the cell suspension into a 15 ml centrifuge tube containing 8 ml of room-temperature culture medium, mixing gently. 6. Centrifuge the mixture at 300 x g for 3 minutes to separate the cells and carefully discard the supernatant containing residual freezing medium. 7. Gently resuspend the cell pellet in 10 ml of fresh culture medium. For adherent cells, divide the suspension between two T25 culture flasks; for suspension cultures, transfer all the medium into one T25 flask to promote effective cell interaction and growth. 8. Adhere to established subculture protocols for continued growth and maintenance of the cell line, ensuring reliable experimental outcomes.
Incubation Atmosphere	37°C, 5% CO_2, humidified atmosphere.
Flask Coating	None
Shipping Conditions	Cryopreserved cell lines are shipped on dry ice in validated, insulated packaging with sufficient refrigerant to maintain approximately −78 °C throughout transit. On receipt, inspect the container immediately and transfer vials without delay to appropriate storage.
Storage Conditions	For long-term preservation, place vials in vapor-phase liquid nitrogen at about −150 to −196 °C. Storage at −80 °C is acceptable only as a short interim step before transfer to liquid nitrogen.

Quality Control & Molecular Analysis

Sterility	Mycoplasma contamination is excluded using both PCR-based assays and luminescence-based mycoplasma detection methods. To ensure there is no bacterial, fungal, or yeast contamination, cell cultures are subjected to daily visual inspections.