WSU-AML, WSU-DLCL2, WSU-FSCCL, WSU-pre-B-ALL and WSU-WM Instructions For Use

Important: Please read these instructions carefully

The viability of these cells is warranted for 30 days from date of shipment when specified reagents and growth conditions are followed as described in this instruction.
If you feel there is a problem with this product, contact Asterand at 1-313-263-0960, 1-866-384-7783 (US Toll Free), +44 (0)1763 211600 (UK), or your sales representative.

These cells are for research use only. Please allow the cells to acclimate before use.

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Product Description

All WSU human cell lines were developed by Drs. Ayad Al-Katib and Ramzi Mohammad at the Barbara Ann Karmanos Cancer Institute of Wayne State University.

WSU-AML: An aggressive human AML cell line established from the frozen peripheral blood of a patient with AML. The cell line showed a partial interstitial deletion of the long arm (q) of chromosome 9. Phenotypically, the cell line is consistent with a diagnosis of M7 AML.

WSU-DLCL2: A human diffuse large cell lymphoma cell line that is a mature B-cell line (IgG lambda) which is negative for EBV nuclear antigen and expresses the multidrug resistance phenotype. The cell line also has t (14; 18)(q32; q21) as well as other chromosomal aberrations.

WSU-FSCCL: A human B-cell line established from an EBV-negative, low-grade follicular, small, cleaved cell lymphoma in leukemic phase.

WSU-pre-B-ALL: A human EBV negative, pre-B-acute lymphoblastic leukemia established from the frozen peripheral blood of a patient with ALL in leukemic phase. Both the frozen cells and the established cell line exhibit the t (1; 19) chromosomal translocation, partial deletion of the short arm (p) of chromosome 9 and a loss of chromosome 13 as clonal abnormalities.

WSU-WM: A human cell line established from the pleural effusion of a patient with IgMλ Waldenstrom's macroglobulinema (WM), a human B-cell tumor.

Quality Control

The cells are grown in antibiotic-free medium and monitored for bacterial contamination. The cell cultures have tested mycoplasma-negative.

Contents and Storage

One vial of 1×10^6 cells in cryopreservation media (CryoStor® CS5, Sigma Aldrich).

Handling Procedures

Biosafety Level 2 safety procedures recommended when handling this cell line.

Required Materials

Reagent	Recommended Supplier	Part Number
RPMI 1640	Gibco	11875
Fetal Bovine Serum (Qualified)	Gibco	26140-079
TrypLE™ Express (*or trypsin*)	Gibco	12605-010

Materials
Cell Culture Treated Flask, T-25
Cell Culture Treated Flask, T-75
70% Ethanol (EtOH)
Sterile Conical Tubes, 50ml

Equipment
Biological Safety Cabinet (BSC) - Level II
Centrifuge
Incubator: 37°C, 5% CO_2
Water bath, 37°C

Culture Medium Preparation

Culture Medium:

1. In Biological Safety Cabinet (BSC)
   a. Prepare reagents (refer to manufacturer instructions for recommended protocols).
   b. Prepare the Culture Medium according to the recipe listed in Table 1 below.
2. Store prepared Culture Medium at 2-8°C until ready for use.

Table 1: WSU-AML, WSU-DLCL2, WSU-FSCCL, WSU-pre-B-ALL and WSU-WM Culture Medium

Component	Stock Concentrations	Final Concentrations	Amount added to 500ml
RPMI 1640	-	-	450ml
Fetal Bovine Serum (Qualified), **heat-inactivated**	-	10%	50ml

Antibiotic/Antimycotic usage: Asterand does not recommend the use of antibiotics or antimycotics. Use in cell culture media at your own discretion.

Cell Thaw

Note: *Some liquid nitrogen-stored vials may blow off cap when transferred to warm water due to gas overexpansion. Always wear appropriate protective clothing when handling frozen vials and perform the following steps as directed.*

1. Equilibrate Culture Medium to 37°C.
2. In BSC, transfer 25ml of Culture Medium to a 50ml conical tube.
3. Using sterile technique, twist cell vial cap one quarter turn. Retighten cap.
4. Quickly swirl and thaw vial in 37°C water bath (~2 minutes). Do not submerge vial past cap threads. Immediately remove vial from bath the moment thaw is complete. **Do not allow the suspension to warm**.
5. Disinfect vial with 70% ethanol (or equivalent) and place in BSC.

Cell Culture

1. In BSC:
   a. Quickly transfer thawed contents from cell vial into the 50ml conical containing pre-warmed Culture Medium and rinse pipette tip 3-5 times.
   b. Optional: rinse vial with Culture Medium to collect any remaining cells and transfer to 50ml conical.
   c. Mix entire suspension thoroughly.

2. Centrifuge the cell suspension at approximately 200xg for 10 minutes.
3. After centrifugation is complete, transfer conical tube to BSC:
    a. Remove supernatant.
    b. Re-suspend cells with 2-3mL of pre-warmed Culture Medium
    c. Remove sample for counting and viability testing (approximately 20µl).
    d. Transfer cell suspension to appropriately sized culture flask and add media as indicated:

Table 2: Initial cell seeding density and media volume

Flask	Media Volume	Total # Cells (Cells/ml)
T-25	5ml	$1x10^6$ ($2x10^5$/ml)
T-75	15ml	$3x10^6$ ($2x10^5$/ml)

4. Gently rock the culture flask to evenly distribute the cells.
5. Place flask into a 37°C incubator at 5% CO_2.
6. Incubate for 1 day then perform Cell Maintenance steps (below).

Cell Maintenance

1. Equilibrate Culture Medium to 37°C.
2. Determine cell concentration by counting cells (refer to Appendix A Cell Culture Images for visual reference).

Note: *Cells grow in suspension* as single cells or aggregates of cells. Separate cell aggregates between subculturing by gently pipetting the suspension up and down until the clumps are reduced in size or eliminated. After initial thaw, cells take approximately 7-10 days to reach maximum concentrations. Thereafter, the cells typically reach maximum concentration in approximately 2-4 days.*
******Some cells may attach to the growth surface of the flask and can be dislodged by moderately rapping the flask or by enzyme dissociation using TrypLE™ Express (or trypsin).*

    a. If cell concentration is less than $1x10^6$ cells/ml, perform steps 3-5.
    b. If cell concentration is $1-2x10^6$ cells/ml, proceed to Cell Subculturing steps.
3. In BSC:
    a. Transfer cell suspension to sterile conical tube(s).
    b. Remove sample for counting and viability testing (approximately 20µl).
    c. Centrifuge the cell suspension at approximately 200xg for 10 minutes.
    d. Add appropriate amount of warmed Culture Medium (refer to Tables 2 and 3).
4. Place flask into a 37°C incubator at 5% CO_2.
5. Incubate cells; observe daily and repeat Cell Maintenance steps as necessary.
6. Change or add additional media 3 times per week to maintain concentration ranges for established cultures.

Cell Subculturing

1. Equilibrate Culture Medium, TrypLE™ and serum-free isotonic solution (e.g. phosphate buffered saline or equivalent) to 37°C.
2. In BSC:
    a. Transfer cell suspension to a sterile conical tube(s).
    b. Rinse flask with 3-5ml pre-warmed serum-free isotonic solution to collect residual cells.
    c. Add 2-5ml fresh, pre-warmed TrypLE™ (if needed to detach adherent cells).
3. Incubate at 37°C, checking for cell dissociation every 2 minutes, until cells are detached.
4. Once cells have detached, transfer flask to BSC and add a volume of pre-warmed Culture Medium equal to that of the TrypLE™ used (to neutralize TrypLE™).
5. Aspirate and pipette cell suspension a number of times to obtain a single-cell suspension.
6. Transfer the suspension to a 50ml conical tube(s).
7. Rinse the flask with an additional 3-5ml of Culture Medium to collect residual cells.

8. Pipette and thoroughly mix the suspension in the conical tube(s).
9. Perform Cell Culture steps 2-6.
    a. Passage cells every 2-4 days as indicated in Table 3.
    b. Refer to Tables 2 and 3 for flask and cell seeding volumes and densities.

Table 3: Cell Line Seeding and Maximum Densities for established cultures

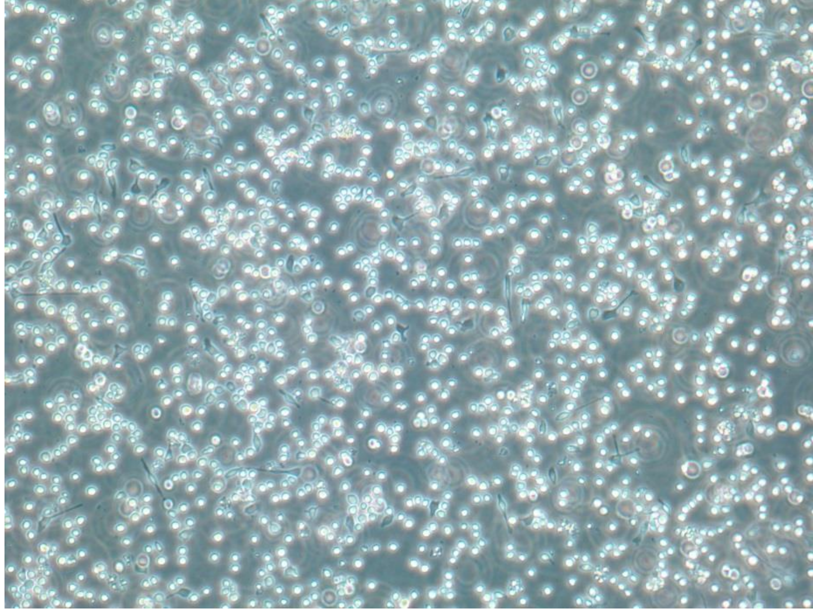
Cell Line	Seeding Density	Maximum Density
WSU-AML	$2x10^5$/ml	$2x10^6$/ml
WSU-DLCL2	$2x10^5$/ml	$2x10^6$/ml
WSU-FSCCL	$2x10^5$/ml	$2x10^6$/ml
WSU-pre-B-ALL	$2x10^5$/ml	$2x10^6$/ml
WSU-WM	$2x10^5$/ml	$2x10^6$/ml

Freezing Cells

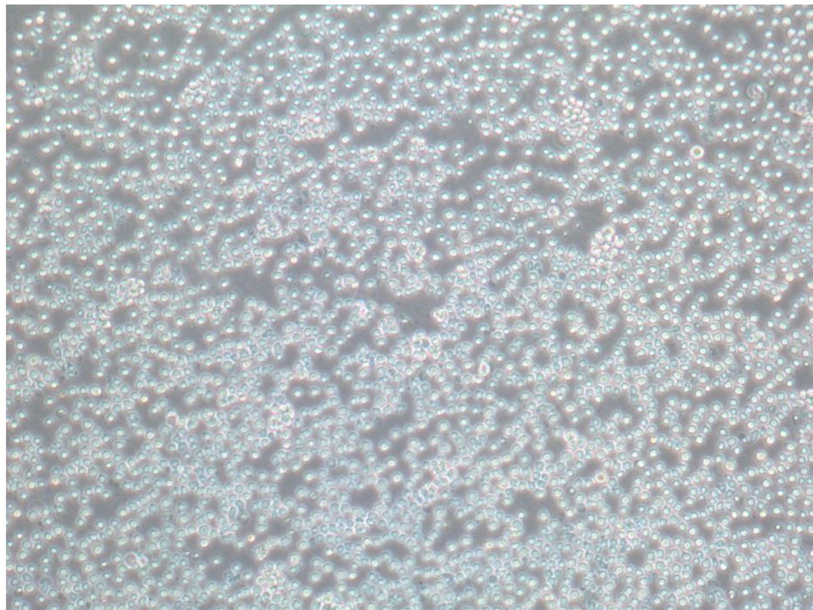
1. Place a controlled rate freezing unit (eg. Nalgene® Mr. Frosty) at 4°C 1-2 hours prior to expected usage.
2. Perform Cell Subculturing steps 1-8.
3. Proceed to perform Cell Culture steps 2 & 3 (a-c).
4. When cell counts/ml of suspension has been determined, centrifuge the suspension again at 200xg for 10 minutes.
5. After centrifugation is complete, transfer conical tube to BSC.
    a. Remove supernatant.
    b. Gradually add cooled (4°C) cryopreservation medium (CryoStor® 5 or preferred cryopreservation medium) to re-suspend the pelleted cells to the desired concentration.
6. Mix to a homogenous suspension and aliquot to cryopreservation vials.
7. Place vials on wet ice for 5-10 minutes then transfer to the pre-cooled controlled rate freezing unit.
8. Place the controlled rate freezing unit in -80°C freezer for 24 hours.
9. Transfer vials of cells from -80°C to liquid nitrogen vapor phase.

APPENDIX A: Cell Culture Images

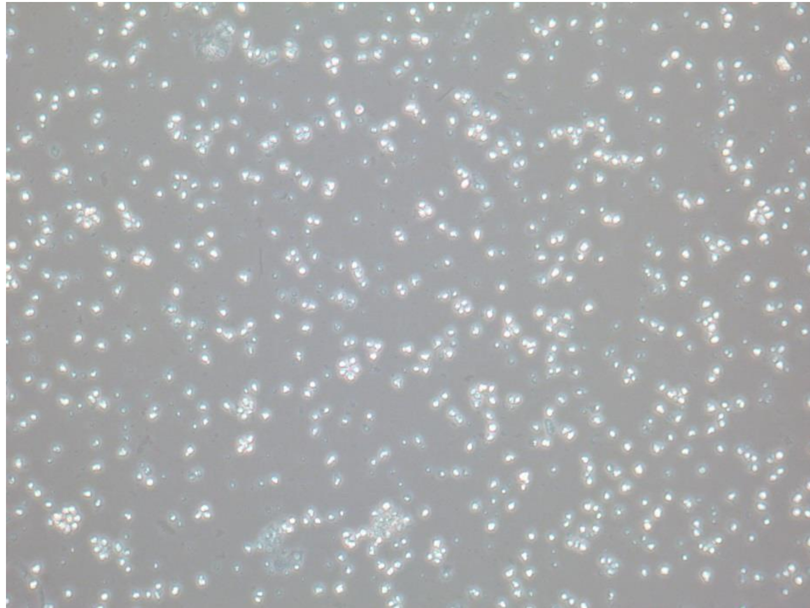
Picture 1: WSU-AML cells in culture



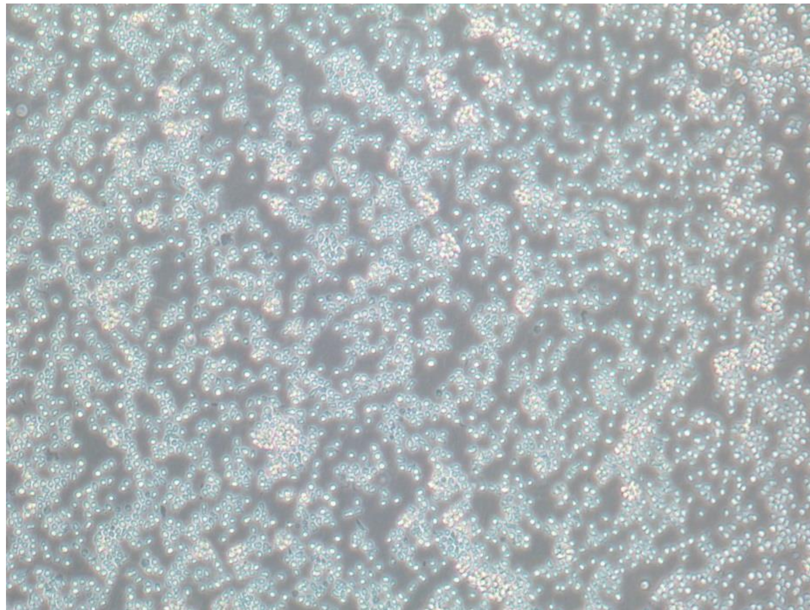
Picture 2: WSU-DLCL2 cells in culture



Picture 3: WSU-FSCCL cells in culture



Picture 4: WSU-pre-B-ALL cells in culture



Picture 5: WSU-WM cells in culture