CHILDREN'S ONCOLOGY GROUP

TEXAS TECH UNIVERSITY HEALTH SCIENCES CENTER
School of Medicine
Cancer Center

SMS-LHN Cell Line Data Sheet

Cell Line Name: **SMS-LHN**

Disease: Neuroblastoma
Phase of Therapy: Post-Chemotherapy (Progressive Disease)
Prior Therapy: Doxorubicin, cyclophosphamide
Disease Stage: 4
Source of Culture: Solid tumor (femur)
Primary Tumor Site: Femur
Date Established:

MYCN Status: Non-amplified
p53 Status: Functional
TH expression: Expressed
Karyotype:
Modal No: 44

IC90 (DIMSCAN*):
***see reference 7**

CBDCA (µg/ml)	CDDP (µg/ml)	DOX (ng/ml)	ETOP (ng/ml)	L-PAM (µg/ml)
1.4	0.1	23.7	559.6	0.5

CBDCA, carboplatin; CDDP, cisplatin; DOX, doxorubicin; ETOP, etoposide; L-PAM, melphalan

Gender: Male
Age: 24 months
Race:

STR Profile: On file at http://strdb.cogcell.com

Media Formulation: Cells are grown in a base medium of RPMI-1640 plus the following supplements (to a final concentration): 10% Fetal Bovine Serum, 2mM L-Glutamine

Growth Conditions: 37.0°C, 5% CO_2, 20% O_2
Subculturing: Next page or Protocols section at http://www.cogcell.org/protocols.php
Doubling Time: 216 hours
Morphology: Teardrop-shaped cells with neurite outgrowth
Growth Properties: Adherent, grows in clusters

Notes:

All COG Repository cell lines are antibiotic-free, mycoplasma-free, and cryopreserved in 50% FBS / 7.5% DMSO. Each vial label contains the cell line name, passage number, total viable cell count (usually 5-10e6), the overall cell viability, and date frozen. All cell lines are validated with original patient sample by STR analysis.

SMS-LHN Cell Line Data Sheet

Cell Line Name: **SMS-LHN**

GROWING CELLS FROM FROZEN CULTURES

To ensure that cells are recovered at the highest viability possible, immediately process cells and place them in culture. If the cells cannot be processed immediately, store the frozen vial in liquid nitrogen vapor (-196°C) upon receipt. Storing cells at -80°C will decrease the viability of the cells.

1. Thaw the vial in a water bath (37°C) for approximately two minutes. To prevent contamination, place the vial in a small ziplock bag or be sure to keep the O-ring and cap out of the water. Thawing should take no longer than 2-3 minutes.
2. While the vial is thawing, place 9 mL of the recommended complete cell culture medium in a centrifuge tube.
3. After the vial has thawed, record the label contents prior to processing. Spray the outside of the vial with 70% ethanol. Remove the contents of the vial and place it in the centrifuge tube containing the complete medium. Spin the tube at 500 x g for 5-7 minutes.
4. After spinning, decant the supernatant and resuspend the pellet in 13 mL of the complete medium. Transfer the cell suspension to a 75 cm^2 culture flask. Incubate the cells at 37°C in 5% CO_2 and room oxygen tension (~20-21% O_2).

MEDIUM RENEWAL

Protocols for medium renewal and subculturing are for a 75 cm^2 flask. If using different sized flasks or other cell culture vessels, adjust volumes accordingly. Cells must be renewed with new medium or subcultured at least every 3-5 days. If cells are not ≥80% confluent after 3-5 days, replace the medium with fresh medium and incubate until they are ready to be subcultured.

1. Remove supernatant from flask and place it in a 15 mL centrifuge tube. Spin the medium at 500 x g for 5 to 7 minutes.
2. After spinning, resuspend the pellet in 13 mL of complete medium and place the suspension back into the 75 cm^2 flask. Be sure to slowly place the medium back into the flask as to not disturb attached or loosely attached cells.

SUBCULTURING CELLS

1. Remove supernatant from flask and place it in a 50 mL centrifuge tube. Add 10-13 mL of Puck's EDTA (see formulation below) to the flask. Be sure the solution is completely covering the plastic surface. Place the flask in the incubator for 10-15 minutes.
2. After incubation, ensure attached cells have detached by gently hitting or shaking the flask. If cells are difficult to detach, incubate flask for 5-10 minutes longer or use a cell scraper to remove remaining cells.
3. When the cells have detached, remove cell suspension and combine with the supernatant medium previously placed in the 50 mL centrifuge tube. (If a cell count is required, cells can be counted after this step). Spin the tube at 500 x g for 5-7 minutes.
4. Decant supernatant and resuspend cells in fresh complete medium and add the appropriate aliquots of the cell suspension to new culture vessels. Seed cells at a ratio of 1:3 to 1:6.
5. Incubate the cells at 37°C in 5% CO_2 and 20% O_2.

PRESERVING CELLS

Cells should be frozen in a mixture of 50% FBS, 7.5% DMSO, and base medium (with no supplements) and stored in liquid nitrogen vapor.

PUCK'S EDTA

Puck's EDTA can be made using the following cell culture grade chemicals at defined concentrations: 140 mM NaCl, 5 mM KCl, 5.5 mM Glucose, 4 mM $NaHCO_3$, 13 µM Phenol Red, 0.8 mM EDTA, and 9 mM HEPES.
The solution should be sterile filtered (0.22 µm) before use. The optimal pH for the solution is a range between 7.2-7.3.

SMS-LHN Cell Line Data Sheet

Cell Line Name: **SMS-LHN**

References:

1. Keshelava N, Zuo JJ, Chen P, Waidyaratine SN, Luna MC, Gomer CJ, Triche TJ, Reynolds C P: Loss of p53 function confers high-level multi-drug resistance in neuroblastoma cell lines. Cancer Res. 61:6185-6193, 2001. PubMed ID: 11507071

2. Reynolds CP, Brodeur GM, Tomayko MM, Donner L, Helson L, Seeger RC, Triche TJ: Biological classification of cell lines derived from human extra-cranial neural tumors. *Prog Clin Biol Res.*271:291-306, 1988. PubMed ID: 3406003

3. Wada RK, Seeger RC, Brodeur GM, Rayner SA, Tomayko MM, Reynolds CP: N-myc expression in human neuroblastoma cell lines without N-myc gene amplification, *Cancer.* 72:3346-3354, 1993. PubMed ID: 8242562

4. Wang Y, Einhorn P, Triche TJ, Seeger RC, Reynolds CP. Expression of Protein Gene Product 9.5 and Tyrosine Hydroxylase in Childhood Small Round Cell Tumors. *Clin Cancer Res.* 6, 551-558, 2000. PubMed ID: 10690538

5. Keshelava N, Groshen S, Reynolds CP. Cross-resistance of topoisomerase I and II inhibitors in neuroblastoma cell lines. Cancer Chemoth Pharm. 45: 1-8, 2000. PubMed ID: 10647494

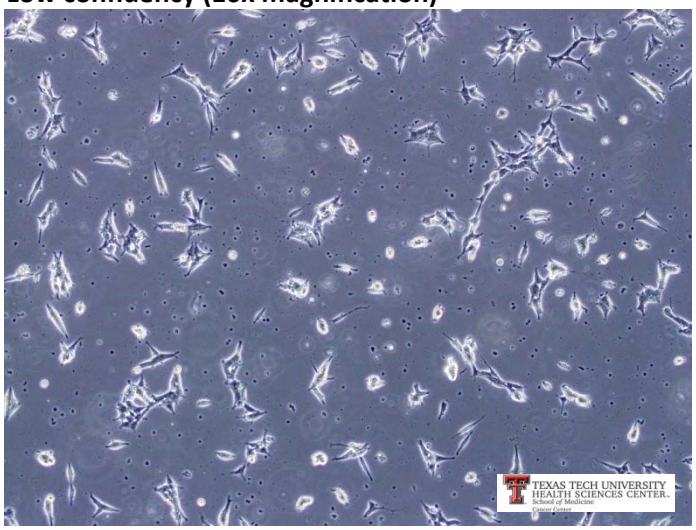
6. Keshelava N, Davicioni E, Wan Z, Ji L, Sposto R, Triche TJ, Reynolds CP. Histone Deacetylase 1 Gene Expression and Sensitization of Multidrug-Resistant Neuroblastoma Cell Lines to Cytotoxic Agents by Depsipeptide. *J Natl Cancer I.* 99: 1107-19, 2007. PubMed ID: 17623797

7. Kang MH, Smith MA, Morton CL, Keshlava N, Houghton PJ, Reynolds CP. National Cancer Institute Pediatric Preclinical Testing Program: Model Description for In Vitro Cytotoxicity Testing. *Pediatr Blood Cancer*. 56: 239-249, 2011. PubMed ID: 20922763 (www.PPTPinvitro.org)

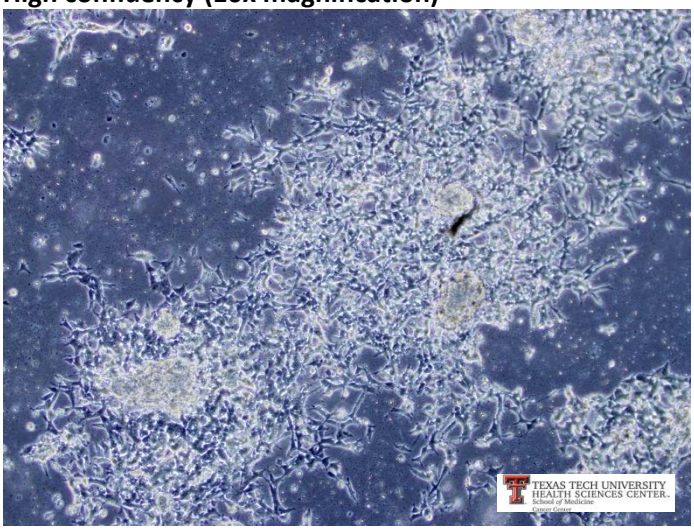
SMS-LHN Cell Line Data Sheet

Cell Line Name: **SMS-LHN**

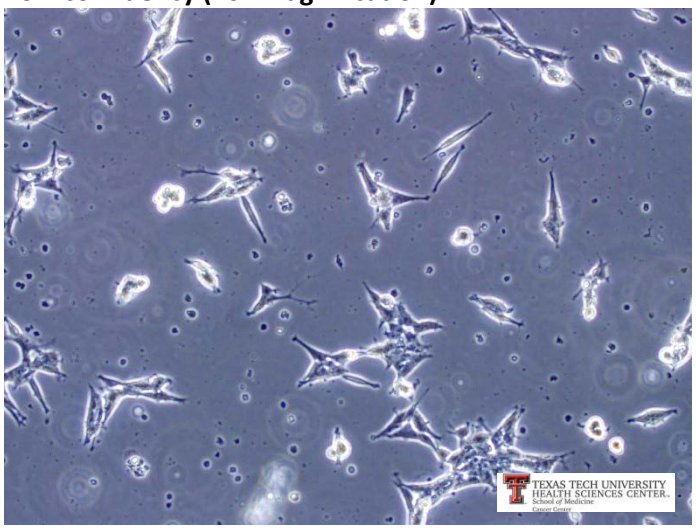
Low confluency (10x magnification)



High confluency (10x magnification)



Low confluency (20x magnification)



High confluency (20x magnification)