Data Sheet

BCRJ Code:	0369
Cell Line:	NCCIT
Species:	Homo sapiens
Vulgar Name:	Human
Tissue:	Embryo, placenta
Morphology:	Epithelial
Disease:	Pluripotent embryonal carcinoma; teratocarcinoma.
Growth Properties:	Adherent
Sex:	Male
Age/Ethinicity:	Adult / Japanese
Tumor Formation::	Yes, Tumors developed within 21 days at 100% frequency (5/5) in nude mice inoculated subcutaneously with 107 cells.
Biosafety:	1
Addtional Info:	This pluripotent stem cell line is capable of somatic and extraembryonic differentiation. The undifferentiated cells are equivalent to a stage intermediate between seminoma and embryonal carcinoma. They will differentiate in response to retinoic acid. NCCIT cells are negative for keratin. They are positive for vimentin and placental alkaline phosphatase.
Culture Medium:	RPMI-1640 medium modified to contain 2 mM L-glutamine, 4500 mg/L glucose with fetal bovine serum to a final concentration of 10%.

Subculturing:	Remove spent medium, add fresh 0.25% trypsin, 0.03% EDTA solution, rinse and remove trypsin. Let the culture sit at room temperature (or at 37°C) for 2 to 5 minutes. Add fresh medium, aspirate and dispense into new flasks. Subculture two times weekly. NOTE: For more information on enzymatic dissociation and subculturing of cell lines consult Chapter 12 in Culture of Animal Cells, a manual of Basic Technique by R. Ian Freshney, 6th edition, published by Alan R. Liss, N.Y., 2010.
Subculturing Medium Renewal:	Add fresh medium at the time of subculture
Subculturing Subcultivation Ratio:	1:4 to 1:8
Culture Conditions:	Atmosphere: air, 95%; carbon dioxide (CO2), 5% Temperature: 37°C
Cryopreservation:	95% FBS + 5% DMSO (Dimethyl sulfoxide)

Thawing Frozen Cells:	SAFETY PRECAUTION: It is strongly recommended to always wear protective gloves, clothing, and a full-face mask when handling frozen vials. Some vials may leak when submerged in liquid nitrogen, allowing nitrogen to slowly enter the vial. Upon thawing, the conversion of liquid nitrogen back to its gas phase may cause the vial to explode or eject its cap with significant force, creating flying debris. 1. Thaw the vial by gently agitating it in a 37°C water bath. To minimize contamination, keep the O-ring and cap out of the water. Thawing should be rapid (approximately 2 minutes). 2. Remove the vial from the water bath as soon as its contents are thawed and decontaminate it by dipping in or spraying with 70% ethanol. From this point, all operations must be performed under strict aseptic conditions. 3. For cells sensitive to DMSO, it is recommended to remove the cryoprotective agent immediately. Transfer the vial contents to a centrifuge tube containing 9.0 mL of complete culture medium and centrifuge at approximately 125 × g for 5 to 7 minutes. 4. Discard the supernatant and resuspend the cell pellet in the recommended complete medium (see specific batch information for the appropriate dilution ratio). 5. Incubate the culture under appropriate atmospheric and temperature conditions (see "Culture Conditions" for this cell line). NOTE: It is important to avoid excessive alkalinity of the medium during cell recovery. To minimize this risk, it is recommended to place the culture vessel containing the growth medium in the incubator for at least 15 minutes before adding the vial contents. This allows the medium to stabilize at its normal pH (7.0 to 7.6).
References:	Teshima S, et al. Four new human germ cell tumor cell lines. Lab. Invest. 59: 328-336, 1988. PubMed: 2842544 Damjanov I, et al. Retinoic acid-induced differentiation of the developmentally pluripotent human germ cell tumor-derived cell line, NCCIT. Lab. Invest. 68: 220-232, 1993. PubMed: 7680083
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