Data Sheet

BCRJ Code:	0022
Cell Line:	4T1
Species:	Mus musculus
Vulgar Name:	Mouse/Balb/Cfc3H
Tissue:	Mammary Gland
Morphology:	Epithelial
Disease:	Stage Iv Human Breast Cancer.
Growth Properties:	Adherent
Derivation:	4T1 is a 6-thioguanine resistant cell line selected from the 410.4 tumor without mutagen treatment.
Applications:	The tumor growth and metastatic spread of 4T1 cells in BALB/c mice very closely mimic human breast cancer. This tumor is an animal model for stage IV human breast cancer. 4T1-induced tumors can be used as a post-operative model as well as a non-surgical model because the 4T1-induced tumor metastasizes spontaneously in both models with similar kinetics
Tumor Formation::	Yes, forms tumors and metastasizes in BALB/c mice
Biosafety:	1
Addtional Info:	When injected into BALB/c mice, 4T1 spontaneously produces highly metastatic tumors that can metastasize to the lung, liver, lymph nodes and brain while the primary tumor is growing in situ. The primary tumor does not have to be removed to induce metastatic growth. Because 4T1 is resistant to 6-thioquanine, micro-metastatic cells (as few as 1) can be detected in many distant site organs with better accuracy that most tumor models. There is no need to count nodules or weight target organs.

Culture Medium:	RPMI-1640 medium modified to contain 2 mM L-glutamine, 1 mM sodium pyruvate, 4500 mg/L glucose and fetal bovine serum to a final concentration of 10%.
Subculturing:	NOTA: Never allow culture to become completely confluent. Remove medium, and rinse with PBS without calcium and magnesium. Remove the solution and add an additional 1 to 2 mL of trypsin-EDTA solution. Allow the flask to sit at room temperature (or at 37°C) until the cells detach. Add fresh culture medium, aspirate and dispense into new culture flasks. 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:	Every 2 to 3 days
Subculturing Subcultivation Ratio:	1:6 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:	49687: Pulaski BA, et al. Immunotherapy with vaccines combining MHC class II/CD80+ tumor cells with interleukin-12 reduces established metastatic disease and stimulates immune effectors and monokine induced by interferon gamma. Cancer Immunol. Immunother.
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