

## Data Sheet

PAGE 1/3

<b>BCRJ Code:</b>	0022
<b>Cell Line:</b>	4T1
<b>Species:</b>	Mus musculus
<b>Vulgar Name:</b>	Mouse/Balb/Cfc3H
<b>Tissue:</b>	Mammary Gland
<b>Morphology:</b>	Epithelial
<b>Disease:</b>	Stage Iv Human Breast Cancer.
<b>Growth Properties:</b>	Adherent
<b>Derivation:</b>	4T1 is a 6-thioguanine resistant cell line selected from the 410.4 tumor without mutagen treatment.
<b>Applications:</b>	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
<b>Tumor Formation::</b>	Yes, forms tumors and metastasizes in BALB/c mice
<b>Biosafety:</b>	1
<b>Additional Info:</b>	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-thioguanine, micro-metastatic cells (as few as 1) can be detected in many distant site organs with better accuracy than most tumor models. There is no need to count nodules or weight target organs.

## Data Sheet

PAGE 2/3

**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 (CO<sub>2</sub>), 5% Temperature: 37°C

**Cryopreservation:**

95% FBS + 5% DMSO (Dimethyl sulfoxide)

**Thawing Frozen Cells:**

**SAFETY PRECAUTION:** It is highly recommended that protective gloves and clothing always be used and a full face mask always be worn when handling frozen vials. It is important to note that some vials leak when submerged in liquid nitrogen and will slowly fill with liquid nitrogen. Upon thawing, the conversion of the liquid nitrogen back to its gas phase may result in the vessel exploding or blowing off its cap with dangerous force creating flying debris. 1. Thaw the vial by gentle agitation in a 37°C water bath. To reduce the possibility of contamination, keep the Oring and cap out of the water. Thawing should be rapid (approximately 2 minutes). 2. Remove the vial from the water bath as soon as the contents are thawed, and decontaminate by dipping in or spraying with 70% ethanol. All of the operations from this point on should be carried out under strict aseptic conditions. 3. For cells that are sensitive to DMSO is recommended that the cryoprotective agent be removed immediately. Transfer the vial contents to a centrifuge tube containing 9.0 mL complete culture medium and spin at approximately 125 x g for 5 to 7 minutes. 4. Discard the supernatant and Resuspend cell pellet with the recommended complete medium (see the specific batch information for the culture recommended dilution ratio). 5. Incubate the culture in an appropriate atmosphere and temperature (see "Culture Conditions" for this cell line). **NOTE:** It is important to avoid excessive alkalinity of the medium during recovery of the cells. It is suggested that, prior to the addition of the vial contents, the culture vessel containing the growth medium be placed into the incubator for at least 15 minutes to allow the medium to reach 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.

**Depositors:**

LUIS RODOLPHO TRAVASSOS; UNIFESP

**ATCC:**

CRL-2539