Data Sheet

Mouse Mammary Tumor TUBO Cell Line

Cancer Cell Line

SCC222

Pack Size: ≥ 1 x 10^6 viable cells/vials

Store in Liquid Nitrogen

FOR RESEARCH USE ONLY

Not for use in Diagnostic Procedures. Not for Human or Animal Consumption.

Background

Breast cancer is the most common malignancy in women throughout the world. The global incidence rate has been increasing annually at a rate of 3.1%.[2] Globally, about 2.1 million women were diagnosed with new occurrences of breast cancer and more than 600,00 women died as a result of breast cancer in 2018. Early diagnosis has been shown to result in a more positive prognosis, making genetic variation and risk factors promising targets for reducing morbidity and mortality.

HER2/neu is a gene implicated in diverse cancers that is particularly important in breast cancer. It is a part of the epidermal growth factor receptor family (EGFR).[3] The HER2 protein product consists of a p185 tyrosine kinase growth factor receptor that is homologous to other EGFRs. When overexpressed or mutated, the extracellular domain will often form homodimers or heterodimers with other EGFRs.[1] Overexpression has typically been correlated with poorer prognosis, higher invasiveness, and can be used to predict the response of certain treatments. HER2/neu gene amplification or overexpression is present in 25-30% of invasive breast carcinomas.

The involvement of p185 in tumor progression and initiation has made it a target of interest for therapy. The TUBO cell line expresses the xenogeneic rat p185 protein.[1] In the rat p185 protein, a single point mutation results in the transformation of the Her-2/neu protooncogene into a dominant oncogene due to homo- and heterodimerization. The TUBO cell line has also been shown to express MHC class I glycoproteins, but not MHC class II. The use of the TUBO mouse cell line enables further research into the mechanisms of breast cancer development and the potential for new therapies or treatments.

Note: Products in this document can be purchased at SigmaAldrich.com using the catalog numbers in parenthesis unless otherwise noted.

Source

The TUBO cell line is a cloned cell line that was established *in vitro* from a lobular carcinoma that was spontaneously produced in a BALB-neuT mouse.[1]

Short Tandem Repeat (STR) Profile

M18-3: 17	M1-2: 17	M8-1: 13	M11-2: 17, 18	MX-1: 25
M4-2: 21.3	M7-1: 24.2, 29	M2-1: 15, 16	M17-2: 16	M13-1: 16.2
M6-7: 12	M1-1: 15	M15-3: 22.3	M12-1: 16	
M19-2: 14	M3-2: 14	M6-4: 17	M5-5: 14	

Cancer cell lines are inherently genetically unstable. Instability may arise in the form of loss of heterozygosity of alleles at one or more genetic sites with increased passages.

Quality Control Testing

- TUBO mouse mammary cells are verified to be of mouse origin and negative for human, rat, Chinese hamster, Golden Syrian hamster, and nonhuman primate interspecies contamination, as assessed by a Contamination Clear panel by Charles River Animal Diagnostic Services.
- Cells are tested negative for infectious diseases by a Mouse Essential CLEAR Panel by Charles River Animal Diagnostic Services.
- Cells are negative for mycoplasma.

Storage and Handling

TUBO mouse mammary cells should be stored in liquid nitrogen until use. The cells can be cultured for at least 10 passages after initial thawing without significantly affecting the cell marker expression and functionality.

‘Representative Data

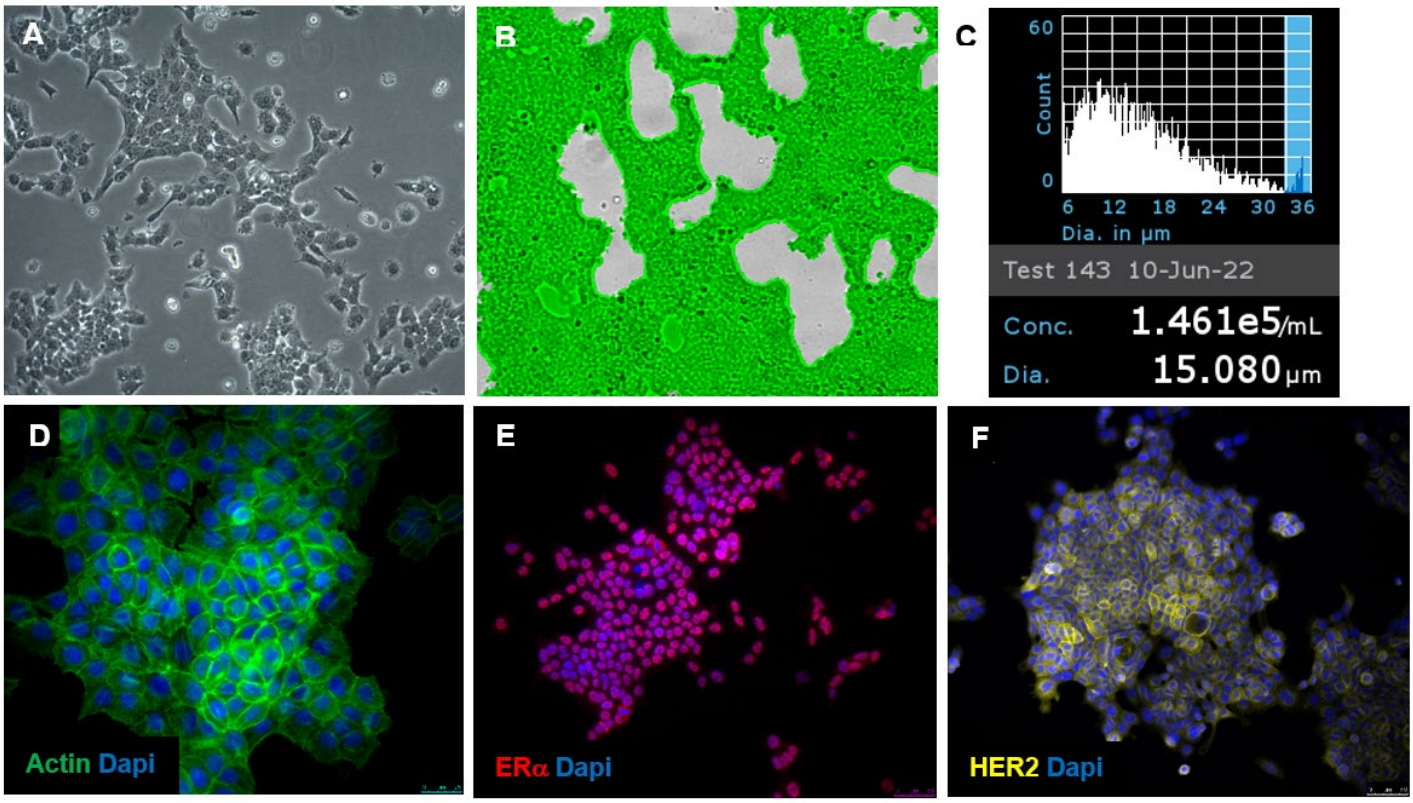

Figure 1: A. Brightfield image of TUBO cells. B. Cell confluency was assessed throughout the culture using the Millicell® Digital Cell Imager (MDCI10000). C. Cell counting was performed using the Scepter™ 3.0 Handheld Automated Cell Counter using 60 µm sensors (PHCC360KIT). D. Cells express actin (49409), E. estrogen receptor alpha (06-935) and F. HER2 (MA513675, Thermo Fisher Scientific).

Protocol

Thawing Cells

1. Do not thaw the cells until the recommended medium is on hand. Cells can grow on standard tissue cultureware surfaces without any additional coating.

   Cells are thawed and expanded in TUBO Expansion Medium comprising DMEM-High Glucose Medium (D6429) containing 20% FBS (for example, ES-009-B), and 2 mM L-Glutamine (TMS-002-C).
2. Remove the vial of frozen TUBO cells from liquid nitrogen and incubate in a 37 °C water bath. Closely monitor until the cells are completely thawed. Maximum cell viability is dependent on the rapid and complete thawing of frozen cells.

   IMPORTANT: Do not vortex cells.
3. As soon as the cells are completely thawed, disinfect the outside of the vial with 70% ethanol. Proceed immediately to the next step.
4. In a laminar flow hood, use a 1 mL or 2 mL pipette to transfer the cells to a sterile 15 mL conical tube. Be careful not to introduce any bubbles during the transfer process.
5. Using a 10 mL pipette, slowly add dropwise 9 mL of TUBO Expansion Medium (Step 1 above) to the 15 mL conical tube.

   IMPORTANT: Do not add the entire volume of media all at once to the cells. This may result in decreased cell viability due to osmotic shock.
6. Gently mix the cell suspension by slowly pipetting up and down twice. Be careful not to introduce any bubbles.

   IMPORTANT: Do not vortex cells.
7. Centrifuge the tube at 300 x *g* for 2-3 minutes to pellet the cells.
8. Decant as much of the supernatant as possible. Steps 5-8 are necessary to remove residual cryopreservative (DMSO).
9. Resuspend the cells in 15 mL of TUBO Expansion Medium.
10. Transfer the cell mixture to a T75 tissue culture flask.
11. Incubate the cells at 37 °C in a humidified incubator with 5% CO_2.

Subculturing Cells

1. Do not allow the cells to grow to confluency. TUBO cells should be passaged at ~70-80% confluency.
2. Carefully remove the medium from the T75 flask containing the 80% confluent layer of TUBO cells.
3. Rinse the flask with 10 mL 1X PBS. Aspirate after the rinse.
4. Apply 5-10 mL of Accutase® or Trypsin/EDTA solution and incubate in a 37 °C incubator for 3-5 minutes.
5. Inspect the flask and ensure the complete detachment of cells by gently tapping the side of the flask with the palm of your hand.
6. Add 5 mL of TUBO Expansion Medium to the plate.
7. Gently rotate the flask to mix the cell suspension. Transfer the dissociated cells to a 15 mL conical tube.
8. Centrifuge the tube at 300 x *g* for 3-5 minutes to pellet the cells.
9. Discard the supernatant, then loosen the cell pellet by tapping the tip of the tube with a finger.
10. Apply 2-5 mL of TUBO Expansion Medium to the conical tube and resuspend the cells thoroughly. Large clumps may be broken up by gentle trituration.

    IMPORTANT: Do not vortex the cells.
11. Count the number of cells using a hemocytometer or a Scepter™ 3.0 Handheld Automated Cell Counter.
12. Plate the cells to the desired density. Typical split ratio is 1:6.

Cryopreservation of Cells

TUBO cells may be frozen in TUBO Expansion Medium supplemented and 10% DMSO using a Nalgene™ slow freeze Mr. Frosty® container.

References

1. *J Immunol* 2000,165(9): 5133-5142.
2. *Nat Rev Dis Primers* 2019, 5(1): 66.
3. *Prog Mol Biol Transl Sci.* 2018, 160: xi-xii. doi: 10.1016/S1877-1173(18)30143-1.

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