Data Sheet

BCRJ Code:	0315
Cell Line:	MAC-T
Species:	Bos taurus
Vulgar Name:	Cow, Bovine
Tissue:	Udder
Cell Type:	Epithelial
Morphology:	Epithelial
Growth Properties:	Adherent
Applications:	Development of the MAC-T bovine mammary epithelial cell line by stable transfection with simian virus-40 large T-antigen should greatly assist study of possible intrinsic (local) and extrinsic (systemic) factors regulating bovine mammary epithelial cell development, differentiation, and function.
Biosafety:	2
Addtional Info:	MAC-T cells were established from primary bovine mammary alveolar cells by stable transfection with SV-40 large T-antigen. MAC-T cells show a population doubling time of approximately 17 h and have been cultured more than 350 passages without showing any sign of senescence. They show the characteristic "cobblestone" morphology of epithelial cells when grown on plastic substratum. Differentiation was induced by augmenting cell-cell interaction on a floating collagen gel in the presence of prolactin.
Culture Medium:	Dulbecco's Modified Eagle's Medium (DMEM) modified to contain 2 mM L-glutamine, 4500 mg/L glucose, 5 ug/mL insulin, 1 ug/mL hydrocortisone and 10% of fetal bovine serum.

Subculturing:	Remove and discard culture medium. Briefly rinse the cell layer with PBS without calcium and magnesium to remove all traces of serum that contains trypsin inhibitor. Add 2.0 to 3.0 mL of Trypsin-EDTA solution to flask and observe cells under an inverted microscope until cell layer is dispersed (usually within 5 to 15 minutes). Note: To avoid clumping do not agitate the cells by hitting or shaking the flask while waiting for the cells to detach. Cells that are difficult to detach may be placed at 37°C to facilitate dispersal. Add 6.0 to 8.0 mL of complete growth medium and aspirate cells by gently pipetting. Add appropriate aliquots of the cell suspension to new culture vessels. Incubate cultures at 37°C. Population doubling time of approximately 17 hours. 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
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:	Huynh HT1, Robitaille G, Turner JD. Exp Cell Res. 1991 Dec;197(2):191-9. Rejman JJ1, Oliver SP, Muenchen RA, Turner JD. Cell Biol Int Rep. 1992 Oct;16(10):993-1001.
Depositors:	MÔNICA MARIA OLIVEIRA PINHO CERQUEIRA - Universidade Federal de Minas Gerais
Cellosaurus:	CVCL_U226