

Data Sheet

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BCRJ Code:	0342
Cell Line:	B16F10-NEX2
Species:	Mus musculus
Vulgar Name:	Mouse; C57BL/6
Morphology:	Fibroblast
Growth Properties:	Adherent
Sex:	Male
Age/Ethnicity:	8 Week /

Derivation:

The cells were isolated from primary tumors developed subcutaneously 20 days after inoculation of 5 x 10e5 cells subclone B16F10-Nex2. The B16F10-Nex2 subclone was obtained from B16F10 cell line obtained from the Ludwig Institute for Research on Cancer (São Paulo Branch). There were no significant changes in the method of growing the subclone in vitro compared to the original cells.

Tumor Formation:: yes

Products: MELANIN

Biosafety: 1

Additional Info:

Typical murine melanoma cell with stelar morphology, heavily melanotic, capable of forming subcutaneous primary tumor and lung metastasis in syngeneic animals. In vitro analysis: cells with high potential of proliferation and invasion and high levels of proliferation. The reisolated cell line from a black tumor was obtained by PhD student Carlos figueireido from the experimental oncology unit of federal university of sao paulo under professor's luiz travassos guidance.

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Culture Medium:	Dulbecco's Modified Eagle's Medium (DMEM) modified to contain 2 mM L-glutamine, 4500 mg/L glucose and fetal bovine serum to a final concentration of 10%.
Subculturing:	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:	1:10
Culture Conditions:	Atmosphere: air, 95%; carbon dioxide (CO ₂), 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:

Polonelli L, Ponton J, Elguezabal N, Moragues MD, Casoli C, Pilotti E, et al. Antibody complementarity-determining regions (CDRs) can display differential antimicrobial, antiviral and antitumor activities. *PLoS One* 2008; 3:e2371. Dobroff AS, Rodrigues EG, Juliano MA, Friaca DM, Nakayasu ES, Almeida IC, et al.: Differential antitumor effects of IgG and IgM monoclonal antibodies and their synthetic complementarity-determining regions directed to new targets of B16F10-Nex2 melanoma cells. *Transl Oncol* 2010, 3:204-17. Arruda DC, Santos LC, Melo FM, Pereira FV, Figueiredo CR, Matsuo AL, et al. b-Actin-binding complementarity-determining region 2 of variable heavy chain from monoclonal antibody C7 induces apoptosis in several human tumor cells and is protective against metastatic melanoma. *J Biol Chem* 2012, 287:14912-22. Massoka MH, Matsuo AL, Scutti JAB, Arruda DC, Rabaça A, Figueiredo CR, et al. Melanoma: perspective of a vaccine based on peptides. In: M.Riese editor. *Molecular Vaccines*. Heidelberg, Springer, 2013.

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Cellosaurus:

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