BCRJ Code:	0342
Cell Line:	B16F10-NEX2
Species:	Mus muscullus
Vulgar Name:	Mouse; C57BL/6
Morphology:	Fibroblast
Growth Properties:	Adherent
Sex:	Male
Age/Ethinicity:	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
Addtional 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.
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 (CO2), 5% Temperature: 37°C
Cryopreservation:	95% FBS + 5% DMSO (Dimethyl sulfoxide)
Thawing Frozen Cells:	SAFETY PRECAUTION: Is highly recommend 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 submersed 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 a 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:

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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