Product description

Third generation cell line derived from a contralateral inguinal lymph node metastasis of a second-generation cell line.

Name: NBF0LN3-384IR Cell Line

Disease: Cancer

Cancers detailed: Melanoma

Tissue: Skin (lymph node metastasis)

Organism: Mouse

Gender: Female

Growth properties: Adherent

Model: Tumorigenic cell line; syngeneic

Conditional: No

Parental cell line: B16-F0

Production details: Line was isolated from an inguinal lymph node metastasis after roughly one month of growth of a LN2 tumour. A luciferase transgene was integrated into the B16-F0 parental line but is no longer expressed in LN metastatic progeny. WES suggests the presence of BRAF and PTEN mutations. These lines are syngeneic in the C57Bl/6 mouse background.

Morphology: Mixed morphology, predominantly spindle-shaped

CRISPR Edited: No

Biosafety level: 1

Application: Syngeneic transplantable models of melanoma metastasis

Mycoplasma free: Yes

Contributor(s)

Inventor: Nathan E. Reticker-Flynn
Institute: Stanford University

Properties

Product format: Frozen

Unpacking and storage:

1. Check all containers for leakage or breakage.

2. Remove the frozen cells from the dry ice packaging and immediately place the cells at a temperature below -130°C, preferably in liquid nitrogen vapor, until ready for use.

Recommended medium: DMEM (high glucose) + 10% FBS + 4mM L-glutamine (or GlutaMAX) + 1% Pen/Strep (optional).

Subculture: Split confluent cultures 1:6 to 1:10 using 0.25% Trypsin-EDTA for 1 min at 37.0°C.

Culture conditions: 37.0°C ± 1.0°C incubator with 5.0% ± 1.0% CO_2

Cryopreservation medium: DMEM, 50% FBS, 10% DMSO

Handling instructions

1. Please ensure that vials are frozen when received, and store at **<-130 °C long term**. When removing frozen cells from storage, it is important to minimize exposure to room temperature (15 - 25°C). If not proceeding directly to thawing, place the cells on dry ice or in a liquid nitrogen container.
2. **Do not thaw at room temperature.** To thaw, swirl the vial quickly in a 37 °C water bath with O-ring and cap above the water to avoid contamination. Remove from the water bath with a small ice pellet remaining (this should not take more than 2 minutes) and wipe the exterior with 70% ethanol or isopropanol before transferring to a biosafety cabinet. Further steps should be conducted under aseptic conditions.
3. We strongly recommend that the volume of cell suspension is measured at this point, and a 20 uL aliquot be removed for a **viable cell count** using trypan blue or similar dye. This ensures that provided cells are viable, and the cell count can be used to determine volume of growth medium to be added to the cell suspension.
4. Transfer the remaining cell suspension to a centrifuge tube using a pipette.
5. Rinse the vial with 1 mL of medium and add it dropwise to the cells. Wash by adding 10 mL of medium dropwise, while gently swirling the tube.
6. Centrifuge the cell suspension at 140 x g for 5 minutes at room temperature. Carefully remove the supernatant with a pipette, leaving a small amount of medium to ensure the cell pellet is not disturbed. Resuspend the cell pellet by gently flicking the tube.
7. Gently add required volume of pre-warmed culture medium, transfer to a suitable cell culture flask and incubate.

Notes:

1. Cell death will be observed post-thaw. It is recommended that cells be thawed in the morning and growth medium is replaced at the end of the day, if necessary.
2. Always culture for a minimum of two passages after thawing before experimental use.
3. Accutase™ is recommended to prepare cells for injections, but Trypsin also works. When preparing cells for injection, use DMEM (without serum or phenol red) and keep the cells at room temperature. Inject 200µL of cell suspension at a concentration of 10^6 cells/mL.

References

- Reticker-Flynn, N.E., et al. (2022). 185(11):1924-1942.e23. PMID: 35525247

Material Citation

If use of this material results in a scientific publication, please cite the material in the following manner: NBF0LN3-384IR Cell Line was invented by Nathan E. Reticker-Flynn (CancerTools.org #162228).