Product description

A novel syngeneic KrasG12D.Smad4+/- murine lung cancer cell line (X577) with orthotopic growth potential, that can serve as a useful preclinical model for evaluating tumour-host interactions, the impact of specific oncogenic alterations on the tumor microenvironment, and immunotherapeutic approaches. This cell line was derived from the primary lung tumour of a KrasLSL-G12D/+.Smad4fl/+ male C57BL/6 mouse and can form orthotopic tumours in immunocompetent C57BL/6 mice. X577 cells demonstrate genetic recombination at the Kras and Smad4 loci as shown by PCR, and express KRASG12D but not SMAD4, as shown by western analysis.

Name: KrasG12D.Smad4+/- murine lung cancer cell line

Organism: Mouse

Gender: Male

Tissue: Lung

Disease: Cancer

Cancer: Lung cancer

Growth properties: Adherent

Production details: A 6 week old KrasLSL-G12D/+.Smad4fl/+ male mouse was injected with 2 µl of 10^10 PFU/mL Ad5-CMV-Cre into the left lung. When this animal was euthanized 26 week later, a 12 mm primary tumour was passaged into a male recipient animal. After 3 passages through recipient animals (two of which included small lung tumours), the KrasG12D.Smad4+/- cell line (X577) was established that was capable of forming orthotopic tumours in immunocompetent C57BL/6 animals.

Application: 2D cell culture; Immunohistochemistry (IHC); Cell line-derived xenograft (CDX) model development

Biosafety level: 1

Contributor(s)

Inventor: Stephen Malkoski
Institute: The University of Colorado Anschutz Medical Campus

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: Dulbecco's Modified Eagle Medium (DMEM) containing 4.5 g/L d-glucose, L-glutamine, and sodium pyruvate, supplemented with 10% (v/v) fetal bovine serum and 100 µg/mL of primocin (optional).

Subculture: 0.25% Trypsin-EDTA at RT for 2-10 minutes or 37°C if especially hard to detach. Split 1:5 to 1:15 when 90% confluent.

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

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 cell suspension into a 15 mL sterile conical tube containing 5 mL of pre-warmed, complete growth medium. Centrifuge cells at **125xg for 5-7 minutes**.
5. Aspirate the supernatant without disturbing the cell pellet. Re-suspend the cell pellet in the recommended pre-warmed, complete growth medium and dispense into a T25 culture flask.
6. Seed at ~20,000 to 50,000 cells/cm^2 (seed more densely at initial thaw - cells can tolerate more sparse subcultures once proliferating consistently) incubate the cells at the recommended conditions.

References

- Nolan et al. 2020. Cancer Cell Int. 28: 20:417. PMID: 32874131

Material Citation

If use of this material results in a scientific publication, please cite the material in the following manner: KrasG12D.Smad4+/- murine lung cancer cell line, was invented by Stephen Malkoski at The University of Colorado Anschutz Medical Campus (CancerTools.org #162384)