Product description

The KPC cell line is derived from the spontaneous tumour of a genetically engineered mouse bearing the LSL-Kras(G12D), LSL-Trp53(R172H), and Pdx-1-Cre alleles, which develop pancreatic ductal adenocarcinoma (PDAC). This cell line models aggressive pancreatic cancer with features resembling human PDAC. It is suitable for cancer biology studies, drug testing, and immune-oncology research.

Name: KPC Cell Line (C57/BL6 genetic background)

Alternate name: PDAC cell line

Disease: Pancreatic cancer

Cancers detailed: Pancreatic cancer; Pancreatic ductal adenocarcinoma

Tissue: Pancreas (tumour)

Tool sub type: Continuous

Organism: Mouse

Gender: Male

Growth properties: Loosely adherent

Model: Transgenic

Conditional: No

Production details: The KPC cell line was established from a spontaneous tumor in a genetically engineered mouse with LSL-Kras(G12D), LSL-Trp53(R172H), and Pdx-1-Cre alleles. These cells are from the KPC mouse which has been backcrossed to F10 in C57/BL6. KPC Subtype: C57Bl6/J. For each cell line, a 5 mm diameter piece of tissue was harvested from solid PDAC arising in a C57BL/6 KPC mouse into DMEM. The tissue was chopped into a fine paste, resuspended in DMEM + L-Glut, Pen-Strep and 20% FBS, and plated out in a 25 cm flask. The medium was changed after 2 days. Once confluent the cells were passaged as normal and grown in DMEM + L-Glut, Pen-Strep and 10% FBS in normoxic conditions at 37 °C in a humidified incubator.

Genotype: *Pdx1-Cre*$^{+}$; *LSL-Kras*$^{G12D/+}$; *LSL-Trp53*$^{R172H/+}$

CRISPR Edited: No

Biosafety level: 2

Contributor(s)

Inventor: Jennifer Morton
Institute: Cancer Research UK, Glasgow Beatson Institute

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 with 10% FBS and 2 mM L-Glutamine.

Subculture: Split 1:2 every 2 days, 0.25% trypsin, 2-5 min at 37.0°C.

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

Cryopreservation: Cells can be cryopreserved in 10% DMSO and 90% FBS or in cell culture medium with 10% DMSO and 10% FBS. It is recommended to freeze 3 vials of 1 mL each from a T75 flask at 80-90% confluency for optimal density and revivability upon thaw.

Protocol for detection of recombined alleles:

- Kras G12D: https://jacks-lab.mit.edu/protocols/genotyping/kras_cond.html
- p53 R172H: https://jacks-lab.mit.edu/protocols/genotyping/p53_recomb.html

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 50 mL conical tube using a pipette.
5. Rinse the vial with 1 mL of medium and add it dropwise to the cells, while gently swirling the 50 mL tube. Wash by adding 15 - 20 mL of medium dropwise, while gently swirling the tube.
6. Centrifuge the cell suspension at 250 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 culture medium and transfer to a suitable cell culture flask and incubate. Examine the cultures after 24 hours and subculture as described.

References

- Sebastiano et al. 2020. Sci. Adv. 6. PMID: 33127675
- Li et al. 2014. Gastroenterology. 146(5):1386-96.e1-17. PMID: 24462734
- Hingorani et al. 2005. Cancer Cell. 7(5):469-83. PMID: 15894267 (**Primary citation**)
- Olive et al. 2004. Cell. 17;119(6):847-60. PMID: 15607980
- Jackson et al. 2001. Genes Dev. 15;15(24):3243-8. PMID: 11751630

Material Citation

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