Product description

NCH1681 is a patient-derived glioma stem-like cell line carrying an IDH mutation, derived from the first relapse of an astrocytoma (WHO grade III). The tumour was obtained from a 38-year-old female patient-derived during surgical resection. The primary tumour was reported as BRAF negative. Cells are cultured under serum-free conditions and grow as free floating neurospheres.

Name: IDH^{mut} glioma stem-like NCH1681 cell line

Cancer type: Brain Cancer

Cancers detailed: Glioma/ secondary glioblastomas

Organism: Human

Tissue: Brain

Growth properties: Neurospheres

Model: Cancer stem cell line

Conditional: No

CRISPR edited: No

Production details: Patient-derived cell line established from human tumour tissue obtained during surgical resection and expanded in vitro.

Cellosaurus ID: CVCL_A5HV

Contributor(s)

Inventor: Christel Herold-Mende
Institute: Heidelberg 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: BIT medium composed of 40 mL DMEM/F-12, 1% GlutaMAX (Gibco™), 10 mL BIT (PELOBiotech), 10 µL FGF-2 (100 µg/mL), 10 µL EGF (100 µg/mL)

Subculture: Only necessary and good for the cells when a high density of spheroids has been achieved and the colour of the medium becomes more yellow. Carefully collect the cell suspension into a 15 mL centrifuge tube. Let cells sediment for 5 min, then remove the supernatant till 1 mL remains. Gently resuspend the cells (approx..20 times) with a glass Pasteur pipette and add fresh BIT medium. Split the cells so that they are still

maintained at a high density. No centrifugation is needed. If it is difficult to obtain a single cell suspension just by mechanical dissociation, incubate the cells after the centrifugation step and after medium removal for 10 minutes with Accutase at 37 °C (1ml / T25 flask and 2ml / T75 flask) before adding fresh BIT medium and resuspending the cells with the glass Pasteur pipette.

Cryopreservation: Obtain a single cell suspension as for passaging. Determine cell count and check for mycoplasma infection. Centrifuge at 300xg for 10 minutes at RT. Discard the supernatant and resuspend the pellet in **synth-a-freeze medium** (per vial: 1 ml medium; 1.5 – 3x10^6 cells). Freeze the vials at -80 °C and transfer them to liquid nitrogen the day after.

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

Handling instructions

Thawing:

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. Prepare sufficient BIT medium at room temperature (T25 flask for 1 x 10^6 cells, T75 flask for up to 5 x 10^6 cells).
3. **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 when the **ice crystals are slightly dissolved** and wipe the exterior with 70% ethanol or isopropanol before transferring to a biosafety cabinet. Further steps should be conducted under aseptic conditions.
4. 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.
5. Add 1 ml of BIT medium to the vial and carefully resuspend.
6. Transfer the cell suspension in a 15 mL tube containing prepared BIT medium.
7. Repeat steps 5 and 6 until the ice crystals have been completely dissolved.
8. Resuspend the cells in BIT medium and transfer them to uncoated, antiadhesive flasks (e.g.Sarstedt).

Maintenance:

1. Let tumour stem cells grow as neurospheres.
2. Cultures must be maintained twice a week.
3. If the mean diameter of the neurospheres is below 150-200 µm or if there are only few neurospheres, only add 1-2 ml of fresh BIT medium (depending on the colour of the medium)
4. If a very low density of tumour stem cells is observed, add a few (2-3) mL of fresh medium to the flask
5. If a higher density of single cells (no spheroids) is observed, exchange of consumed medium is required:
   • carefully collect the cells in a 15 mL centrifuge tube
   • centrifuge at 300xg for 5 minutes at RT
   • discard the supernatant
   • resuspend the pellet in fresh BIT medium and transfer it to the culture flask
6. If a higher density of spheroids is observed, this also requires an exchange of consumed medium:
   • carefully rinse the cell suspension over the bottom of the culture flask and collect it in a 15 mL centrifuge tube
   • let cells sediment for 5 minutes
   • remove the supernatant until 1 mL is remaining
   • add fresh BIT medium to the cells, carefully resuspend and transfer to the culture flask
7. If the diameter of the neurospheres exceeds 200 µm, passage the cells following instructions above.

References

- Dettling et al. 2018. Clin Cancer Res. 24(12):2951-2962. PMID: 29563135.
- Lita et al. 2021. Nat Commun. 12(1):614. PMID: 33504762

Material Citation

If use of this material results in a scientific publication, please cite the material in the following manner: IDH^{mut} glioma stem-like NCH1681 cell line, was invented by Christel Herold-Mende at the University of Heidelberg (CancerTools.org #162304).