Product description

GCGR-E55 is a primary, patient-derived, IDH-wildtype glioblastoma stem-like cell line derived from human tumour tissue. The line grows as an adherent, laminin-dependent monolayer, has a doubling time of approximately 65 hours, and forms tumours in vivo. Early-passage transcriptomic profiling (P3) indicated a mesenchymal-like transcriptional state based on probability scoring. The line shows an injury-associated gene expression phenotype with enrichment for mesenchymal-immune (MESImm) gene programmes.

Name: GCGR-E55 Cell Line

Organism: Human

Gender: Male

Disease: Cancer

Cancer Type: Brain cancer

Cancers detailed: Glioblastoma, IDH wildtype

Tissue: Glioblastoma

Production details: Derived from surgically resected primary glioblastoma tissue and expanded as an adherent monolayer under laminin supported condition

Additional notes: This cell line is a primary glioblastoma stem-like model derived from a single patient tumour and exhibits intrinsic cellular heterogeneity, reflecting the diversity and plasticity typical of glioblastoma. Transcriptional state classifications were assessed at early passage (P3) and may change in culture.

GBM subtype: Mesenchymal, MESImm

Methylation subclass: GBM_RTK_I

Molecular data available: RNA-seq, EPIC methylation arrays, WGS

Tumour initiation: Yes (~4 months)

Approx. doubling time (hr): 65

Growth properties: Adherent

Morphology: Adherent monolayer with stem-like features (SOX2, NESTIN).

Model: Primary cell line

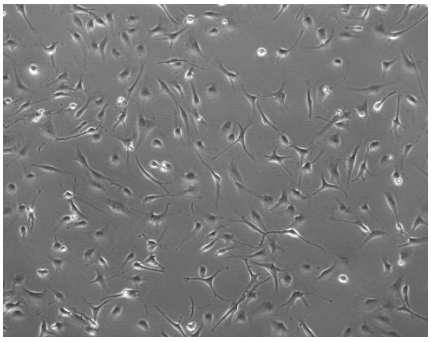
Biosafety level: 2

Mycoplasma free: Yes

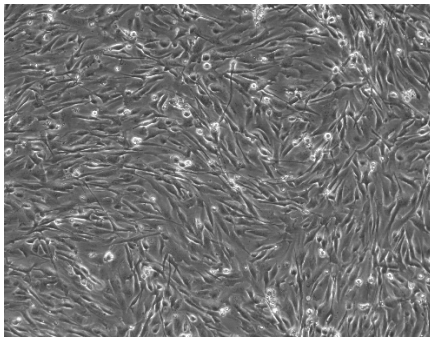
Contributor(s)

Inventor: Steven Pollard and Gillian Morrison
Institute: The University of Edinburgh

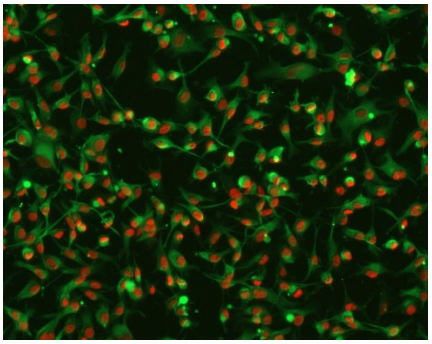
Passage 7, 24 h post thaw (10x)



Passage 7, day 6 (10x)



Anti-NESTIN ab: MAB1259, anti - SOX2 ab: Ab92494



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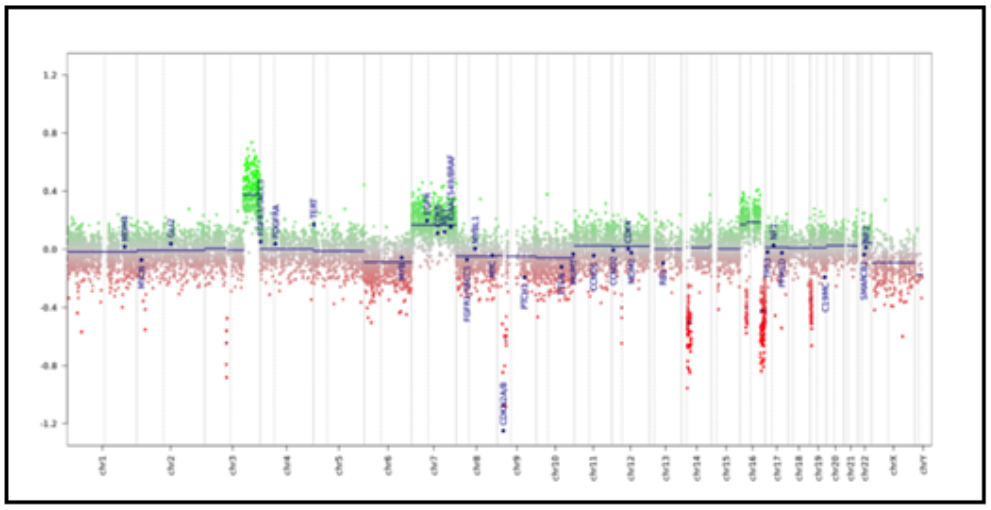

STR profiling

STR locus	genotype
AMEL	X, Y
CSF1PO	10, 12
D13S317	8, 9
D16S539	12
D18S51	12, 15
D21S11	28, 30
D3S1358	16, 17
D5S818	11, 13
D7S820	8, 10
D8S1179	13, 14
FGA	19, 22
PENTA D	9, 12
PENTA E	7, 13
THO1	6
TPOX	8
vWA	14, 19

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.

Plasticware: Corning cell culture flasks (T25: 430639, T75: 430641U) and cryovials (Nunc 377224).

Important note regarding Laminin use: All GCGR GSC lines require laminin as a substrate for optimal growth as adherent monolayer. All lines grow well on laminin pre-coated plastic. However, many of the GSC lines grow as an adherent monolayer without pre-coating by supplementing the media directly with laminin. The dependency of the cell lines on laminin pre-coating for optimal growth may be tested and optimized in-house following receipt of the cells.

Laminin preparation: 5 ml Cultrex® 3-D Culture Matrix Laminin I (6 mg/ml) (R&D Systems 3446-005-01) is thawed overnight at 4ºC, then carefully added to a 50 ml Falcon tube containing 25 ml ice cold PBS and mix gently by inverting. Then aliquot 30 x 1 ml at 1 mg/ml. Work quickly and do not allow laminin to rise in temperature. Store aliquots at -20°C. Thaw aliquots at 4°C, store at 4°C and use within 1 month. Laminin supplied by Sigma (L2020) has also been successfully used at the same concentrations.

Growth Factor preparation:

- rhFGFbasic 500 ug (Peprotech: 100-18b) reconstituted in 5 ml PBS/0.1% BSA = 100 ug/ml. Aliquot 80 x 60ul and store at -20°C short term or -80°C for longer term storage. Thawed aliquots are stable at 4°C for up to 4 weeks.

- rmEGF 500 ug (Peprotech: 315-09) reconstituted in 5 ml PBS/0.1% BSA = 100 ug/ml. Aliquot 80 x 60 ml and store at -20°C short term or -80°C for longer term storage. Thawed aliquots are stable at 4°C for up to 4 weeks.

Laminin coating of cell culture plastic:

Add ice cold laminin stock to ice cold PBS to a final concentration of 10 ug/ml (1:100 dilution of 1 mg/ml stock). Immediately coat the surface of plastic with the Laminin solution and allow to gel for minimum of 2 hr at 37°C. Remove laminin immediately before plating cells, avoiding washing.

Recommended growth medium:

- Complete medium: 500 ml DMEM/HAMS-F12 (Sigma D8437) media with, 7.25 ml Glucose (Sigma G8644), 5 ml MEM NEAA 100x (Gibco 11140-035), 5 ml Pen-Strep (Gibco 15140-122), 800 ul BSA Soln 7.5% (Gibco 15260-037), 1 ml bMercETOH 50mM (Gibco 31350-010) , 5 ml B27 Supplement 50x (LifeTech/Gibco 17504-044) and 2.5 ml N2 Supplement 100x (LifeTech/Gibco 17502-048). Add the N2 supplement last, slowly swirling bottle to avoid precipitation. Complete media can be stored at 4°C up to a month or -20°C for longer.

- Before use, complete media is supplemented with Mouse EGF to final concentration 10 ng/ml, Human FGF-2 to final concentration 10 ng/ml, and Laminin-1 to a final concentration 2 ug/ml. This media (termed, 'Complete+EFL') should be stored at 4°C and used within 4 weeks**.**

- Wash medium: 500ml DMEM/HAMS-F12 (Sigma D8437) media with 5 ml Pen-Strep (Gibco 15140-122) and 1 ml BSA 7.5% (Gibco 15260-037).

- Cryopreservation medium: Wash medium + 10% final concentration of DMSO.

Subculture: Passage cells at 80-90% confluency, split ratio 1:4 to 1:6. Aspirate media and add sufficient Accutase (Sigma A6964 or BioLegend 424201) to cover surface (approx. 1 ml for T25). 37°C, 2-5 minutes. Flasks may be tapped to encourage detachment. Add wash media and pipette cell suspension, centrifuge 3 min @1200 rpm to pellet cells. Approx. doubling time (hr): 65.

Culture conditions: 37.0°C ± 1.0°C 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 minimise 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. **Do not vortex.** Further steps should be conducted under aseptic conditions.
3. Add 10 ml of prewarmed wash media to a 15 ml conical tube.
4. Gently transfer thawed cells to pre-warmed wash media.
5. Centrifuge 3 min @1200 rpm to pellet cells.
6. Aspirate supernatant and resuspend cells in 8 ml of Complete media + EFL and transfer to a T25 flask (or split between 2 wells of a 6 well plate with 1/4 in 1 well and 3/4 in another to avoid any seeding density issues).
7. Replace the media the next day to ensure that all residual DMSO and dead cells are removed.

Additional notes:

- Accutase must be removed by aspiration, dilution is not enough.
- Media should be replaced every 7 days.
- Do not split cells at a too high a ratio. Cells plated at low density often decrease/stop proliferating.
- Cells around the edges of the flask occasionally form spheres. These can detach.
- GCGR GSC lines do not proliferate optimally in flasks larger than T75.
- The growth rate of different GSC lines vary. As a rough guide, an average GSC line requires a 1:6 split every 7-14 days.

References

- Pollard et al. 2009. Cell Stem Cell. 5;4(6):568-80. PMID: 19497285
- Furqan M et al. Pharmaceutics 2025 PMID: 40430842.

Material Citation

If use of this material results in a scientific publication, please cite the material in the following manner: GCGR-E55 cell line was invented by Steven Pollard and Gillian Morrison at the University of Edinburgh (CancerTools.org #140079).