D54 (aka H54) Glioblastoma Cell Line Parameters

1. Source of cells: Duke University Medical Center, surgical resection from a patient with glioblastoma multiforme (WHO Grade IV). D54 is a commonly studied glioblastoma cell line (Bao et al., 2006) that has been thoroughly described by S Bigner (1981). Requests for D54 cells should be directed to Darrell Bigner (bigne001@mc.duke.edu).

2. Lineage of cells: tumor-derived cancer cells. Cell of origin of glioblastoma is the astrocyte.

3. Donor information: 36 year old Caucasian female.


5. Media for cell lines: 1X Zinc Option Media (prepared from Gibco Improved MEM Zinc Option Media 5X Concentrate, formula# 86-0194D). Each liter of medium is supplemented with 10 mL Hepes and 40 mL 5.5% NaHCO₃. Cells are grown in media supplemented with 10% FBS (Gibco Certified FBS, Cat#16000-044) and 2% Pen/Strep.

6. Culture conditions: Cells grow adherent to a plastic dish or flask and should be incubated at 37ºC in the presence of 5% CO₂. At sparse density, cells grow as individual colonies, while at high density (>50% confluence) cells form foci of 10-50 cells piled on top of the surrounding monolayer. Cells are passed when the monolayer reaches ~80% confluence (prominent foci will be formed by this time). Once the cells have reached upwards of 80% confluency they will quickly turn the media orange or yellow, so split them once they reach this point.

7. Cell Line Maintenance: Cells for passage are trypsinized in 0.1% Trypsin/EDTA (diluted from 0.5% Trypsin/EDTA from Gibco, Cat#15400-054). It is not necessary to wash cells prior to trypsinization and the cells should detach within 5-10 min at 37ºC. New dishes can be made with 1/10-1/20 dilutions from near-confluent dishes, resulting in approximately 2-3 day and 4-5 day passages, respectively.

Cells may be frozen at a density of 10⁶ cells/mL in ZO medium supplemented with 50% FBS and 20.5% DMSO. To freeze cells, pellet cells as if passaging, then resuspend in freezing medium and freeze at a rate of 1 ºC/min, then store in the vapor phase of liquid nitrogen. Frozen cells are recovered by thawing in a 37 ºC water bath, washing once with PBS or ZO medium, and resuspending in 8 mL of 20% ZO medium in a T-25 flask. Once the medium has begun to turn orange, replace with 2 volumes of 10% ZO medium and maintain as above.

8. Cell passage#: unknown

References:
