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
4. Karyotype: abnormal
5. Media for cell lines: 1X Zinc Option Media (prepared from Gibco Improved MEM Zinc Option Media 5X Concentrate, formula# 86-0194DJ). Each liter of medium is supplemented with 10 mL Hepes and 40 mL 5.5% $NaHCO_3$. 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 37º in the presence of 5% CO_2. 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 passaged 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 37º. 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 10^6 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:

Bao S, Wu Q, McLendon RE, Hao Y, Shi Q, Hjelmeland AB, Dewhirst MW, Bigner DD, Rich JN. Glioma stem cells promote radioresistance by preferential activation of the DNA damage response. Nature. 2006 Dec 7;444(7120):756-60. Epub 2006 Oct 18.

Bigner SH, Bullard DE, Pegram CN, Wikstrand CJ, Bigner DD. Relationship of in vitro morphologic and growth characteristics of established human glioma-derived cell lines to their tumorigenicity in athymic nude mice. J Neuropathol Exp Neurol. 1981 Jul;40(4):390-409.