**Cell Line**

Cell Line: VL-1

~~Lot number:~~

IMMORTALIZED:

***Working cell name:***  BDF P5 SV40 MOI20 (Lot 15151?)

***—------------------------------------------------------------------------------------------------------------------***

**For baseline and robust characterizations, can enter values in the tables below. Make sure to transfer the values to “cell\_line\_development” database, where each characterization type (baseline or robust) will be a separate row.**

***—------------------------------------------------------------------------------------------------------------------***

***Baseline cell characterization***

| **Characteristic** |  | **Additional notes** |
| --- | --- | --- |
| Population doubling time | 19-23 hours (P18 to P20) | VL1 expansion and banking - PDTs over 3 passages (**MFD081**)  *\*****MFD080*** *had PDTs range from 35-75 hrs in 3 different media formulations.* |
| Population doublings | 3.8 to 3.0 (P18 to P20) | Population doublings over 3 passages (**MFD081**)  *\*****MFD080*** *had PDs range from 2.2-4.2 (hypox.) and 1.7-3.8 (norm.) in 3 different media formulations.* |
| Cell viability | >90% (P18 to P20) | Cell viability over 3 passages (**MFD081**) |
| Cell size | N/A | Individual cell size not captured. **MFD083** provides area of cell (um2) in relation to TGF-ꞵ conc. curve. |
| Cell culture conditions | Old VL1 SEGM: aMEM, 1% hPL, FGF, EGF, epinephrine, AA2P, hydrocortisone, insulin | [VL1 SR Medium Formulations](https://docs.google.com/spreadsheets/u/0/d/1bx3Rv5lSNI_QcnA7ZJnhBMceKpJ8zn_Wx-MUInwnTpk/edit) |

**Decision point:** Advance forward for robust characterization? YES or NO

Rationale: VL1s found to grow in SRGM formulations with a preference for 1% hPL and hypoxia in culture conditions.

***Robust cell characterization***

| **Characteristic** |  | **Additional notes** |
| --- | --- | --- |
| Cell line senescence | N/A | N/A |
| Tissue Induction | ↑ Extracellular CHP observed vs DMEM (10% FBS) | Experiment codes: MFD082, MFD083, MFD086 |
| Contraction potential | N/A | N/A |
| CHP staining | Collagen deposition observed | MFD086 - 2 ng/mL TGF-ꞵ optimal for collagen deposition |
| Culture conditions | [VL1 SR Medium Formulations](https://docs.google.com/spreadsheets/u/0/d/1bx3Rv5lSNI_QcnA7ZJnhBMceKpJ8zn_Wx-MUInwnTpk/edit) |  |

**Decision point:** Advance forward for 3D tissue formation w/ biomaterial? YES or NO

Rationale: VL1 had poor biomaterial attachment in all media, resulting in a lot of cell death. Blebbing was observed during additional SRTM characterization, suggesting that the cells are dying as well.

**NO GO - Cell line abandoned due to previously noted issues identified during characterization studies.**

**Decision point:** Advance forward for additional cell line development? YES or NO

Rationale: **NO GO - Cell line abandoned due to previously noted issues identified during characterization studies.**

***Relevant data (Historical & Revisited):***

*\*Note - Historical recap is a review of previous VL1 cell line work (2020-2022) that made the case to revisit cell line to prove or disprove continued cell line usage.*

*Revisited is the recap of experiments that demonstrated additional cell line issues leading to the discovery of multiple concerning factors for the cell line and rationale for abandoning the cell line completely in this form.*

[VL1 cell line summary](https://drive.google.com/open?id=1ms-iRHrAeQ_onjavBgVTlSXWltId_BCf)

***Cell line transduction/transfection:***

| **Goal / Purpose** | To generate an immortalized cell line that will co-express SV40 LT and puromycin resistance on a lentiviral backbone. | *General description for vector design purpose and function* |
| --- | --- | --- |
| **Sequence Author** | Elson S | *Who designed the vector* |
| **Creation Date** | *Unknown* | *When was the completed vector designed* |
| **Vector Accession Number** | VB200429-2198ctb | *List file name for design* |
| **Working name** | BDF P5 SV40 MOI20 (Lot 15151?) | *Internal R&D name. This is likely to change once moved beyond CLD work.* |
| **Link to vector design** | [VB200429-2198ctb](https://www.dropbox.com/s/bs2ackdfdfiv9qa/VB200429-2198ctb.dna?dl=0) | *Direct link to the Snapview file (Map, Sequence, Enzymes, Features, Primers, History)* |
| **Map image** |  | *Insert image of vector* |
| **Cell line / Passage number** | BDF P5 | *Specific cell line and passage number used for transfection* |
| **Transfection date** | ~ July 2020 | *When was the transfection performed* |
| **Virus MOI** | MOI = 20 | *Note the tested multiplicity of infection (MOI) titer and optimal MOI for virus.* |
| **Cell Selection** | Puromycin | *Note if cell selection was used for modification.* |
| **Kill curve info** | 0.5 ug/mL (?) | *Note the appropriate concentration required to kill untransfected cells. Needs to be established for each cell type and/or each new agent or lot of selective antibiotic is used.* |
| **Experimental outcome** | Transfection appears successful. Concerns about slowing PDT approaching P35-40. Heterogeneity may be a factor. Controlling cell growth may be optimized with MFD. | *Notes on success/failure, possible concerns* |

***Note:*** SnapGene enables an easy and secure way to plan, visualize, and document everyday molecular biology procedures. With an intuitive interface, the software enables DNA sequence visualization, sequence annotation, sequence editing, cloning, protein visualization, and simulating common cloning methods.

