

## Maintenance of OP9 cells

### OP9 Medium

Minimum Essential Medium- Alpha ( $\alpha$ -MEM) powdered media is used (Gibco #12000-022)

The powder is added to 900ml of sterile distilled water while stirring. The package is rinsed out at least twice.

Once the powder has dissolved add the following:

- 2.2g of Sodium Bicarbonate (Tissue culture tested)
- 6ml of L-glutamine (200mM stock, Sigma #G7513)
- 6ml of diluted  $\beta$ -mercaptoethanol\*\*
- 5ml of penicillin\streptomycin solution (Gibco #15140-148, 50ug/ml final conc.)

**\*\*Note:** Diluted 2-mercaptoethanol is made by adding **70ul** of 2-mercaptoethanol (Sigma, M7522) to 100ml of sterile PBS or water.

Top up to 1L with sterile dH<sub>2</sub>O

Filter-sterilize using a 0.22um bottle top filter.

\*Before use add 100ml of FBS to the 500ml bottle.

Thaw the OP9 cells at 37°C and then transfer the contents of the vial to a tube containing 5mls of OP9 media. Spin down the cells at 1000 rpms for 5min. Resuspend the cell pellet in 3mls of OP9 media and transfer to a 10cm plate containing 10ml of OP9 media. Once a confluent plate is achieved, usually 2-3 days of growth, the OP9 cells can be easily maintained by passing them using a 1:3 split every Monday, Wednesday, and Friday. The media does not need to be changed in between passages.

When freezing down OP9 cells you will need to freeze down one confluent plate into 2 vials. We freeze in 40% FBS, 10% DMSO and 50% OP9 media.

Over confluent cells will differentiate into adipocytes producing large vacuole containing cells. If this occurs a new vial must be thawed since they will not go away with passaging and will only get worse. As long as they don't go more than three days between passages and don't get over confluent then they should stay healthy for a long time. If a sheet of cells is still present after five minutes in trypsin at 37°C then incubate a little longer in trypsin and do at least a 1:4 or 1:5 split for the next few passages.