Code:	0309
Cell Line:	OP9
Species:	Mus musculus **Vulgar Name:** Mouse
Tissue:	Bone Marrow/Stroma
Morphology:	Fibroblast-Like
Growth Properties:	Adherent
Age Ethinicy:	newborn
Derivation:	The OP9 cell line was established from newborn op/op mouse calvaria.
Applications:	OP9 cells can be used to coculture mouse embryonic stem cells (ES cells) to induce the differentiation of embryonic stem (ES) cells into blood cells of erythroid, myeloid, and B cell lineages. Cocultivation with OP9 does not require exogenous growth factors or complex embryoid structures. This system will facilitate the study of molecular mechanisms involved in development and differentiation of hematopoietic cells.
Biosafey:	1
Additional info:	The cells do not produce functional macrophage colony-stimulating factor (M-CSF) due to an osteopetrotic mutation in the gene encoding M-CSF. The presence of M-CSF had inhibitory effects on the differentiation of embryonic stem (ES) cells to blood cells other than macrophages
Culture Medium:	Alpha Minimum Essential Medium without ribonucleosides and deoxyribonucleosides and with 2.2 g/L sodium bicarbonate and fetal bovine serum to a final concentration of 20% .
Subculturing:	NOTE: Maintain cultures at a cell concentration between 4 X 10e3 and 1 X 10e4 cells/cm2. Volumes are given for a 75 cm2 flask. Increase or decrease the amount of dissociation medium needed proportionally for culture vessels of other sizes. Note: Cell density is important. If the subculture ratio is too low, the culture will not

	reach confluence. However, do not overgrow. Very large cells tend to appear after overgrowth and these cells are a warning sign that the OP9 cells will not support the maintenance of hematopoietic cells. Subculture just before confluence. Remove and discard culture medium. Briefly rinse the cell layer with PBS without calcium and magnesium to remove all traces of serum which contains trypsin inhibitor. Add 2.0 to 3.0 mL of Trypsin-EDTA solution to flask and observe cells under an inverted microscope until cell layer is dispersed (usually within 5 to 15 minutes). Note: To avoid clumping do not agitate the cells by hitting or shaking the flask while waiting for the cells to detach. Cells that are difficult to detach may be placed at 37°C to facilitate dispersal. Add 6.0 to 8.0 mL of complete growth medium and aspirate cells by gently pipetting. Transfer cell suspension to a centrifuge tube and spin at approximately 125 X g for 5 to 10 minutes. Discard supernatant. Resuspend the cell pellet in fresh growth medium. Add appropriate aliquots of the cell suspension to new culture vessels. Incubate cultures at 37°C. Population Doubling Time: 26 hrs NOTE: For more information on enzymatic dissociation and subculturing of cell lines consult Chapter 12 in Culture of Animal Cells, a manual of Basic Technique by R. Ian Freshney, 6th edition, published by Alan R. Liss, N.Y., 2010. **Medium Renewal:** Every 2 to 3 days **Subcultivation ratio:**
Culture Conditions:	Atmosphere: air, 95%; carbon dioxide (CO_2), 5% Temperature: 37°C
Cryopreservation:	95% FBS + 5% DMSO (Dimethyl sulfoxide)
Thawing Frozen Cells:	SAFETY PRECAUTION: Is highly recommend that protective gloves and clothing always be used and a full face mask always be worn when handling frozen vials. It is important to note that some vials leak when submersed in liquid nitrogen and will slowly fill with liquid nitrogen. Upon thawing, the conversion of the liquid nitrogen back to its gas phase may result in the vessel exploding or blowing off its cap with dangerous force creating flying debris. 1. Thaw the vial by gentle agitation in a 37°C water bath. To reduce the possibility of contamination, keep the Oring and cap out of the water. Thawing should be rapid (approximately 2 minutes). 2. Remove the vial from the water bath as soon as the contents are thawed, and decontaminate by dipping in or

	spraying with 70% ethanol. All of the operations from this point on should be carried out under strict aseptic conditions. 3. For cells that are sensitive to DMSO is recommended that the cryoprotective agent be removed immediately. Transfer the vial contents to a centrifuge tube containing 9.0 mL complete culture medium and spin at approximately 125 x g for 5 to 7 minutes. 4.Discard the supernatant and Resuspend cell pellet with the recommended complete medium (see the specific batch information for the culture recommended dilution ratio). 5. Incubate the culture in a appropriate atmosphere and temperature (see "Culture Conditions" for this cell line). NOTE: It is important to avoid excessive alkalinity of the medium during recovery of the cells. It is suggested that, prior to the addition of the vial contents, the culture vessel containing the growth medium be placed into the incubator for at least 15 minutes to allow the medium to reach its normal pH (7.0 to 7.6).
References:	Nakano T, et al. Generation of lymphohematopoietic cells from embryonic stem cells in culture. Science 265: 1098-1101, 1994.
Depositors:	ANA FLAVIA POPI - ECB
ATCC:	CRL-2749