PROBETEX
Experimental Pathology Resources™

Data Sheet

1 X 10^6 cells

Lot # W501

Mouse Metanephric Mesenchymal Cells (CMMM-1)

Materials supplied:

This package contains 1.0 X 10^6 cells in 1 ml of cryoprotective medium (Basal Eagle's medium with Hank's BSS, 5% FBS, 7.5% DMSO) plus antibiotic/antimycotic.

Description

Probetex mouse metanephric mesenchymal (MMM-1) cells are derived from metanephric mesenchyme at gestational day 11 (Wagner et al, 2007). The cells were dissociated and immortalized by transfection with human papilloma virus. The cells were identified and their purity was assessed by phase-contrast microscopy showing a stellate cell shape (Figure 1) in low density and expression phenotypic markers vimentin (Figure 2), alpha-smooth muscle actin, PDGFR-β by immunofluorescence microscopy. The cells are negative for the ureteric bud epithelial cell markers, EndoA cytokeratin Aquaporin 2, and *Dolichos biflorus*. When combined with mouse ureteric cells (CMUB-1), CMMM-1 cells organize into capillary-like structures and UB cells into cysts and tubuloid arrangements in three-dimensional growth in Matrigel® implanted subcutaneously into SCID mice (Velagapudi et al, 2012). Each stock re-animates with a doubling time of approximately 12 hours.

Directions for use

Please read carefully before starting re-animation.

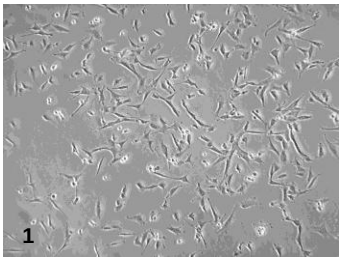
Re-animation: Thaw the cells quickly in a 37°C water bath. Wipe down the outside with 70% ethanol before bringing the vial into your cell culture hood. Transfer the cells to 10 ml of warm growth medium. Centrifuge the cells at ~400 x g for 5 min. Resuspend the cells in a total of 20 ml growth medium (DMEM high glucose, 10% Fetal Bovine Serum, with or without antibiotics) and transfer to a T-75 flask. Incubate at 37°C and 5% CO_2. Replace medium 24-48 hours after re-animation.

Subculturing: Remove the medium from confluent, viable cells. Rinse with Mg^{2+}/Ca^{2+}-free Phosphate-Buffered Saline (PBS). Detach the cells by addition of 0.05% trypsin/EDTA solution and incubate the cells for up to 10 min at 37°C. Add growth medium and collect the cells. Centrifuge the cells at 400 x g for 5 min. Resuspend the cells in fresh growth medium. 10-20-fold split ratios are suggested.

Cryopreservation: Treat the cells the same way as for subculturing. Determine the number of viable cells and resuspend them at 2.0 X 10^6 cells/ml in growth medium. Add an equal volume of cryoprotective medium for a recommended final DMSO concentration of 7.5%. The recommended storage condition is liquid nitrogen vapor phase.

Adventitious Agents:

This product is certified free of bacteria, fungi, and mycoplasma.



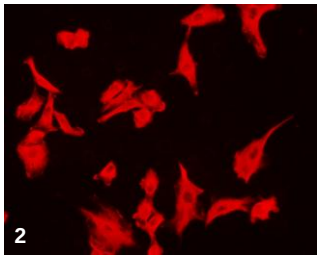


Figure 1. Phase contrast micrograph of CMMM-1 cells showing cells in a stellate shape. Figure 2. Fluorescence microscopy of CMMM-1 displaying a mesenchymal marker vimentin.

Wagner B, Ricono JM, Gorin Y, Block K, Arar M, Riley D: Mitogenic signaling via platelet-derived growth factor b in metanephric mesenchymal cells. J Am Soc Nephrol 18: 2903-2911, 2007.

Velagapudi C, Nilsson R-P, Lee MJ, Burns HS, Ricono JM, Arar M, Barnes VL, Abboud HE, Barnes JL,: Reciprocal induction of simple organogenesis by mouse kidney progenitor cells in three-dimensional co-culture. Am J Pathol 180: 819-830, 2012. See Am J Pathol Cover Image February issue 2012.

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