PROBETEX
Experimental Pathology Resources™

Data Sheet

CMMM Lot # W501 **CMUB Lot #** W508

Mouse Metanephric Mesenchymal Cells (CMMM-1)
Mouse Ureteric Bud Cells (CMUB-1)
for simple organogeneisis in 3-D culture ex vivo *

Materials supplied:

This package contains 2 vials, one each of CMMM and CMUB each with 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

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

CMMM-1: 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*.

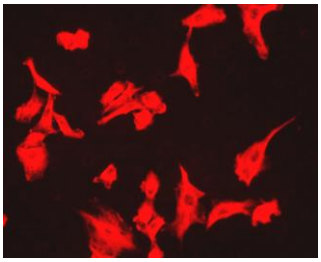
CMUB-1: Probetex mouse ureteric bud (CMUB-1) cells are derived from ureteric bud at gestational day 11 (Ye et al, Kidney Int. 66:1356-1364, 2004). The cells were dissociated and immortalized by transfection with human papilloma virus. The cells were identified and their purity assessed by phase-contrast microscopy showing a stellate shape in low density growth (Figure A) and the immunofluorescence microscopic expression of embryonic epithelial cell marker Endo-A cytokeratin (Figure B), Aquaporin 2, and UB marker *Dolichos biflorus*. The cells are negative for the mesenchymal cell markers vimentin, alpha-smooth muscle actin, and PDGFR-β. The cells form cysts and branching cords in 3-dimensional growth in collagen containing fibronectin (Ye et al.) and form well-defined tubular epithelium when combined with mouse metanephric mesenchymal cells (CMMM-1) in Matrigel® implanted subcutaneously into SCID mice (Velagapudi et al. Am J Pathol180:819-830, 2012).

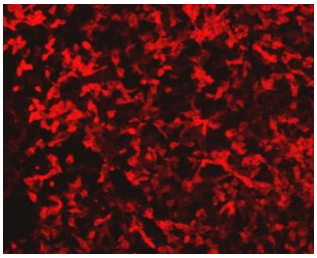
***Simple Organogenesis:** When CMMM-1 and CMUB-1 are combined in three-dimensional growth in Matrigel® implanted subcutaneously into SCID mice, the MM cells organize into capillary-like structures and the UB cells into cysts and tubuloid arrangements with collecting duct markers (Velagapudi et al. Am J Pathol180:819-830, 2012).

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. Each stock re-animates with a doubling time of approximately 12 hours

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 0.25% 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-fold to 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.





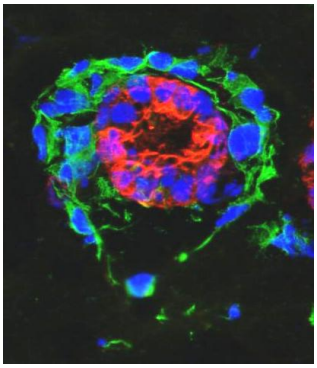


Figure 1. A. CMMM-1 displaying a mesenchymal marker vimentin. B. CMMM-1 displaying a mesenchymal marker vimentin in 2-dimensional culture.

B. CMUB-1 displaying an embryonic epithelial cell marker EndoA cytokeratin in 2-dimensional culture

C. CMMM-1 (aquaporin 1, green) and CMUB-1 (EndoA, red) in 3-dimensional culture in matrigel implants in SCID mice.

Probetex, Inc.
7418 John Smith, Suite A
San Antonio, TX 78229

(210) 616-9515 FAX: (210) 616-9914
e-mail: info@probetex.com