

1 X 10<sup>6</sup> cells

Lot # W499

## Mouse Ureteric Bud Cells (CMUB-1)

### Materials supplied:

This package contains 1.0 X 10<sup>6</sup> 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 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 Pathol* 180:819-830, 2012). Conditioned medium derived from CMUB-1 cells induces CMMM-1 cells to migrate (Velagapudi et al.) and has been reported to induce renal lineage cells to produce MM markers (GDNF, WT1 and Cadherin 11) (Nishikawa et al. *Biochem Biophys Res Comm* 417:897-902, 2012).

### Directions for use (Please read carefully before re-animation).

**Re-animation:** Each stock re-animates with a doubling time of approximately 11 hours. 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<sub>2</sub>. Replace medium 24-48 hours after re-animation.

**Subculturing:** Remove the medium from confluent, viable cells. Rinse with Mg<sup>2+</sup>/Ca<sup>2+</sup>-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- to 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<sup>6</sup> 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:** Free of bacteria, fungi, and mycoplasma.

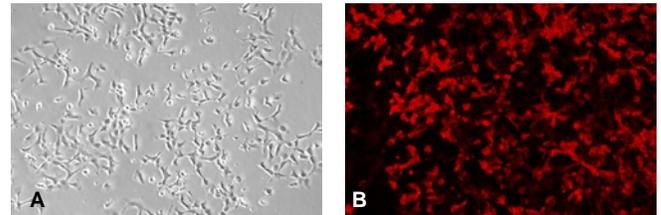


Figure 1. Phase contrast micrograph of CMUB-1 cells showing cells in a stellate shape (A) and displaying an embryonic epithelial cell marker EndoA cytokeratin by fluorescence microscopy (B).

1. Ye P, Habib SL, Ricono JM, Kim N-H, Choudhury GG, Barnes JL, Abboud HE, Arar MY: Fibronectin induces ureteric bud cells branching and cellular cord and tubule formation. *Kidney Int.* 66:1356-1364, 2004.
2. 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 Cover Image, *Am J Pathol* February issue 2012).
3. Nishikawa M, Yanagawa N, Kojima N, Yuri S, Hauser PV, Jo OD, Yanagawa N: Stepwise renal lineage differentiation of mouse embryonic stem cells tracing in vivo development. *Biochem Biophys Res Comm* 417:897-902, 2012.