

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

Lot # W508

## Bovine Glomerular Endothelial Cells (CBGEN-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 bovine renal glomerular endothelial (BGEN-1) cells are derived from a fresh calf kidney obtained from a local abattoir. The cells were cloned from cells obtained from collagenase treated glomeruli (Grandaliano et al). The cells were identified and their purity was assessed by phase-contrast microscopy showing a polygonal cell shape (Figure 1) and immunofluorescence microscopic evaluation of phenotypic markers factor VIII and cellular uptake of fluorescent-labeled 1,1-dioctadecyl-1-1-3,3,3,3-tetramethyl-indocarbocyanine perchlorate acetylated LDL (DiI-Ac-LDL) (Figure 2). The cells are negative for mesangial and epithelial markers. Each stock re-animates with a doubling time of 2 days.

### 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 (RPMI-1640, 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 0.25% 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. 3- to 4-fold split ratios are recommended.

**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:

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

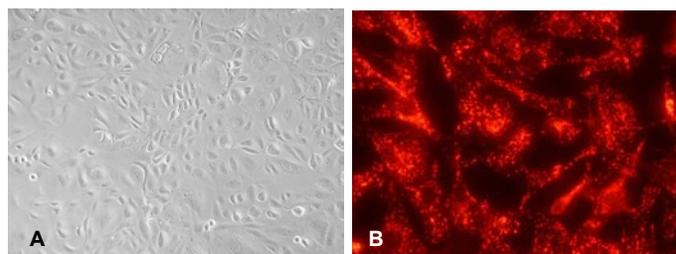


Figure 1. (A) Phase contrast micrograph of CBGEN-1 cells showing cells in a polygonal shape. (B) Fluorescence microscopy of CBGEN-1 cells labeled with DiI-Ac-LDL.

1. Grandaliano G et al: Thrombin regulates PDGF Expression in Bovine Glomerular Endothelial Cells. J Am Soc Nephrol 9:583-589, 1998.
2. Kasinath BS. Glomerular endothelial cell proteoglycans--regulation by TGF-beta 1. Arch Biochem & Biophysics. 305:370-377, 1993.