ProstaCult™ Mouse Medium Kit

Medium for culture of mouse prostate epithelial cells

Catalog #05640 500 mL

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Product Description

ProstaCult™ is a serum-free liquid culture medium for the culture of mouse prostate epithelial cells. It is ideal for the culture and evaluation of mouse prostate epithelial progenitor cells in the prostate colony-forming unit (CFU) assay when used in conjunction with an irradiated feeder layer such as NIH 3T3 cells. Addition of human recombinant epidermal growth factor (EGF), human recombinant basic fibroblast growth factor (bFGF), and heparin is required for culturing cells.

Product Information

The following components are sold as a complete kit (Catalog #05640) and are not available for individual sale.

COMPONENT NAME	COMPONENT #	SIZE	STORAGE	SHELF LIFE
ProstaCult™ Basal Medium	05641	500 mL	Store at 2 - 8°C.	Stable until expiry date (EXP) on label.
ProstaCult™ Proliferation Supplement*	05642	5 mL	Store at 2 - 8°C.	Stable until expiry date (EXP) on label.

*This product contains material derived from human plasma. Donors have been tested and found negative for HIV-1 and -2, hepatitis B, and hepatitis C prior to donation. However, this product should be considered potentially infectious and treated in accordance with universal handling precautions.

Materials Required But Not Included

PRODUCT NAME	CATALOG #
Collagenase/Hyaluronidase	07912
D-PBS Without Ca++ and Mg++ (PBS)	37350
Dispase (5 U/mL)	07913
DMEM/F-12 with 15 mM HEPES	36254
DNase I Solution (1 mg/mL)	07900
Fetal bovine serum (FBS; quality cell culture-tested)	---
Hanks' Balanced Salt Solution with 10 mM HEPES, Without Phenol Red	37150
Heparin Solution	07980
Human Recombinant bFGF	78003
Human Recombinant EGF	78006
Trypan Blue	07050
Trypsin-EDTA (0.25%)	07901
40 µm Cell Strainer	27305

Preparation of Complete ProstaCult™ Medium (Mouse)

Use sterile techniques to prepare complete ProstaCult™ Medium (Mouse) (ProstaCult™ Basal Medium + ProstaCult™ Proliferation Supplement + EGF + bFGF + heparin). The following example is for preparing 500 mL of complete medium. If preparing other volumes, adjust accordingly.

NOTE: Avoid the use of glass pipettes and tubes when handling prostate epithelial cells. These cells will stick to the glass.

1. Add 5 mL of ProstaCult™ Proliferation Supplement to 500 mL of ProstaCult™ Basal Medium.

   NOTE: If not used immediately, store ProstaCult™ Medium at 2 - 8°C for up to 1 week.
2. Immediately before use, add cytokines and heparin to ProstaCult™ Medium as follows:
   - 10 ng/mL Human Recombinant EGF
   - 10 ng/mL Human Recombinant bFGF
   - 4 µg/mL (0.0004%) heparin

Directions for Use

Please read the entire protocol before proceeding.

A. DISSOCIATION OF MOUSE PROSTATE GLANDS

1. Dilute 1 part Collagenase/Hyaluronidase (10X stock) with 9 parts DMEM/F-12 supplemented with 5% FBS and place into a 14 mL or 50 mL centrifuge tube. Approximately 2 - 5 mL of this diluted Collagenase/Hyaluronidase solution will be required for every 2 - 3 mouse prostates to be dissociated.
2. Resect prostates and transfer to a sterile Petri dish containing cold phosphate-buffered saline (PBS). Using a dissecting microscope, scissors, and a fine set of forceps, remove residual amounts of fat from prostate tissue.
3. Transfer prostate tissue to the tube containing the diluted Collagenase/Hyaluronidase solution (see step 1) and incubate at 37°C for 3 hours.
4. Centrifuge cells at 350 x *g* for 5 minutes and discard supernatant.
5. Resuspend pellet in 5 - 6 mL of Trypsin-EDTA (0.25%) and leave on ice for 1 hour.
6. Add 10 mL of cold Hanks' Balanced Salt Solution with 10 mM HEPES, Without Phenol Red supplemented with 2% FBS and centrifuge at 350 x *g* for 5 minutes. The Hanks' + FBS solution will be referred to as HF.
7. Remove as much of the supernatant as possible.
8. Add 2 mL of warm Dispase (5 U/mL) and 200 µL of DNase I Solution (1 mg/mL). Pipette the sample up and down for 1 minute with a 1 mL micropipette.
9. Add 10 mL of cold HF and filter cell suspension through a 40 µm Cell Strainer into a new 50 mL centrifuge tube. Centrifuge at 350 x *g* for 5 minutes with the brake on and discard supernatant. Resuspend cells in a medium suitable for subsequent assays.
10. Count viable cells using Trypan Blue and a hemocytometer.

B. MOUSE PROSTATE COLONY-FORMING UNIT (CFU) ASSAY

Mouse prostate epithelial cell cultures should be initiated from single-cell suspensions obtained from dissociated mouse prostate glands (section A).

NOTE: To generate epithelial colonies, mouse prostate cells should be plated at clonal densities on a pre-established irradiated viable feeder layer. The use of NIH 3T3 cells irradiated at 5 x 10^3 cGy and seeded at 10^4 cells/cm^2 is recommended. Epithelial colonies will be generated after 7 - 10 days when cultured under these conditions.

1. Add mouse prostate cells to tissue culture flasks at a density of 4 - 8 x 10^3 cells/cm^2 in complete ProstaCult™ Medium supplemented with 5% FBS. For a 6-well plate, add 4 - 8 x 10^4 cells per well.

   NOTE: Failure to include serum in the medium will result in poor adherence of the cells to the tissue culture plastic.
2. Incubate at 37°C for 7 - 10 days. On the second day, change the culture medium to serum-free complete ProstaCult™ Medium.

   NOTE: Failure to change the medium to serum-free complete ProstaCult™ Medium could result in overgrowth of the culture by contaminating stromal cells.
3. Fix, stain, and count the CFUs.