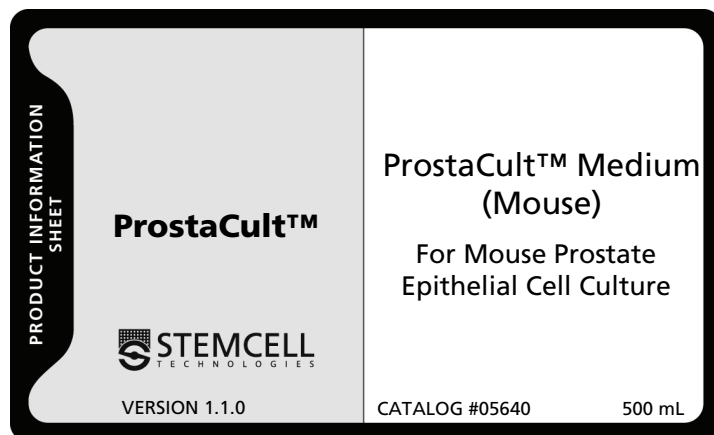


PRODUCT DESCRIPTION

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 progenitors in prostate colony-forming cell assay when used in conjunction with an irradiated feeder layer such as NIH 3T3 cells. Addition of EGF, Human, Recombinant (Catalog #02633), bFGF, Human, Recombinant (Catalog #02634), and Heparin (Catalog #07980) is required for culturing cells.



COMPONENTS

05641	ProstaCult™ Basal Medium	500 mL
05642	ProstaCult™ Proliferation Supplements	5 mL

This product is a biological reagent and as such cannot be completely characterized or quantified. Some variability is unavoidable.

This product has been aseptically manufactured using tightly controlled processes and is sterility tested.

This product contains components derived from human plasma. Venous blood from each donor has been tested for hepatitis B surface antigen (HBsAg) and HIV-1 antibodies and/or HIV-1 antigen. However, this product should be considered potentially infectious and treated in accordance with universal handling precautions.

STABILITY AND STORAGE

05641 ProstaCult™ Basal Medium 500 mL
Product stable at 2 - 8°C for at least one year from date of manufacture as indicated on the label (refer to Directions for Use for more details).

05642 ProstaCult™ Proliferation Supplements 5 mL
Product stable at 2 - 8°C until expiry date as indicated on the label (refer to Directions for Use for more details).

Refer to Material Safety Data Sheet for additional information.

RELATED PRODUCTS

PRODUCT	CATALOG #
40 µm Cell Strainers	27305
Collagenase/Hyaluronidase Solution (10X)	07912
Dispase (5 mg/mL)	07913
DMEM/F-12	36254
DNase I (1 mg/mL)	07900
Fetal Bovine Serum (FBS)	06100/06150
Hanks' Balanced Salt Solution, Modified	37150
Heparin	07980
rh Epidermal Growth Factor (rh EGF)	02633
rh basic Fibroblast Growth Factor (rh bFGF)	02634
Trypan Blue	07050
Trypsin-EDTA (0.25%)	07901

DIRECTIONS FOR USE

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

1.0 Preparation of Complete ProstaCult™ Medium (Mouse)

1. Prepare Complete ProstaCult™ Medium by adding 5 mL thawed ProstaCult™ Proliferation Supplements (Component #05642) to 500 mL ProstaCult™ Basal Medium (Component #05641).
2. Supplement with rh EGF (Catalog #02633) and rh bFGF (Catalog #02634) to obtain a final concentration of 10 ng/mL for each.

Note: EGF and bFGF will lose some activity when combined with medium and stored at 2 - 8°C for more than 2 weeks. It is recommended to add rh EGF and rh bFGF to medium immediately before use.

3. Add Heparin (Catalog #07980) to a final concentration of 4 µg/mL.

Note: When preparing medium for the initial plating of cultures, the addition of 5% FBS (Catalog #06100/06150 or quality cell culture-tested equivalent) is required (refer to Section 3.0, Step 1).

2.0 Dissociation of Mouse Prostate Glands

1. Dilute 1 part 10X Collagenase/Hyaluronidase (Catalog #07912) with 9 parts DMEM/F-12 (Catalog #36254) supplemented with 5% fetal bovine serum (Catalog #06100/06150) and place into a 14 mL or 50 mL centrifuge tube. Approximately 2 - 5 mL of this 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 PBS. Using a dissecting microscope, a fine set of forceps and scissors, remove residual amounts of fat from prostate tissue.
3. Transfer prostate tissue to the tube containing the Collagenase/Hyaluronidase/FBS/DMEM/F-12 solution and incubate for 3 hours at 37°C.
4. After dissociation, centrifuge cells at 350 x g for 5 minutes with the brake on and discard supernatant.
5. Resuspend pellet in 5 - 6 mL of 0.25% Trypsin-EDTA (Catalog #07901) and leave on ice for 1 hour.
6. Add 10 mL cold Hanks' Balanced Salt Solution Modified (Catalog #37150) supplemented with 2% FBS (Catalog #06100) and centrifuge at 350 x g for 5 minutes with the brake on.
7. Remove as much of the supernatant as possible.
8. Add 2 mL prewarmed 5 mg/mL Dispase (Catalog #07913) and 200 µL of 1 mg/mL DNase I (Catalog #07900). Pipette the sample up and down for 1 minute with a disposable plastic tip attached to a 1 mL micropipette.
9. Add 10 mL cold Hanks' Balanced Salt Solution Modified (Catalog #37150) supplemented with 2% FBS (Catalog #06100) and filter cell suspension through a 40 µm cell strainer (Catalog #27305) 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 (Catalog #07050) and a hemacytometer.

3.0 Culture of Mouse Prostate Epithelial Cells

Mouse prostate epithelial cell cultures should be initiated from single cell suspensions obtained from dissociated mouse prostate glands (refer to Section 2.0).

Note: To generate epithelial colonies, mouse prostate cells should be plated at clonal densities (<2000 cells/cm²) and should be performed using pre-established irradiated viable feeder layers. The use of NIH 3T3 cells irradiated at 5 x 10³ cGy and seeded at 10⁴ cells/cm² is recommended. Epithelial colonies will be generated after 7 - 10 days when cultured under these conditions.

1. Plate mouse prostate cells into tissue culture flasks at a density of 4 - 8 x 10³ cells/cm² in Complete ProstaCult™ Medium containing rh EGF (Catalog #02633), rh bFGF (Catalog #02634), Heparin (Catalog #07980) and 5% FBS (Catalog #06100/06150). For example, add 4 - 8 x 10⁴ cells per well of a 6-well plate.

Note: Failure to include serum during plating of prostate cells will result in poor adherence of the cells to the tissue culture plastic.

2. After 24 hours, change the culture medium to serum-free Complete ProstaCult™ Medium supplemented with rh EGF, rh bFGF and Heparin.

Note: Failure to change the medium to serum-free Complete ProstaCult™ Medium could result in overgrowth of the culture by contaminating stromal cells.

NOTES

- **Conversion of g to rpm.** To convert g to rpm, use the following formula:

$$\text{RPM} = \sqrt{\frac{\text{RCF}}{(1.118 \times 10^{-5}) \times (\text{Radius})}}$$

Where: RCF = relative centrifugal force (g)
RPM = centrifuge speed in revolutions/minute
Radius = radius of rotor in cm

REFERENCES

1. Abate-Shen C, Shen MM: Molecular genetics of prostate cancer. *Genes Dev* 14: 2410-2434, 2000
2. Lawson DA, Witte ON: Stem cells in prostate cancer initiation and progression. *J Clin Inv* 117: 2044-2050, 2007
3. Wang Y, Hayward S, Cao M, Thayer K, Cunha G: Cell differentiation lineage in the prostate. *Differentiation* 68: 270-279, 2001
4. Xue Y, Smedts F, Debryne FM, de la Rosette JJ, Schalken JA: Identification of intermediate cell types by keratin expression in the developing human prostate. *Prostate* 34: 292-301, 1998
5. Van Leenders G, Dijkman H, Hulsbergen-van de Kaa C, Ruiters D, Schalken J: Demonstration of intermediate cells during human prostate epithelial differentiation in situ and *in vitro* using triple-staining confocal scanning microscopy. *Lab Invest* 80: 1251-1258, 2000
6. Lawson DA, Xin L, Lukacs RU, Cheng D, Witte ON: Isolation and functional characterization of murine prostate stem cells. *Proc Natl Acad Sci USA* 104: 181-186, 2007