CELL APPLICATIONS, INC.

LIFE TECH Life Technologies™ Your Molecular & Cell Technology Partner

General Instructions for Use

Human Gastrointestinal Epithelial Cells

Instructions apply to Gastric (HGaEpC), Intestinal (HInEpC), Colonic (HCnEpC), Duodenum (HDuEpC), Ileum (HIlEpC), and Jejunum (HJeEpC) Epithelial Cells

Be sure to wear face protection mask and gloves when retrieving cryovials from the liquid nitrogen storage tank. The dramatic temperature change from the tank to the room could cause any trapped liquid nitrogen in the cryovials to burst and cause injury.

Open all the packages immediately upon arrival and examine each component for shipping damage. Notify Cell Applications, Inc. or your distributor immediately if there is any problem.

I. STORAGE

A. CRYOPRESERVED VIALS (732Ga-10, 732In-10, 732Cn-10, 732Du-10, 732Je-10, 732Il-10)

Store the cryovials in a liquid nitrogen storage tank immediately upon arrival.

B. GI EPITHELIAL CELL DEFINED CULTURE MEDIUM (716DC-50)

Store at 4°C in the dark immediately upon arrival. Use within 4 weeks.

C. GI EPITHELIAL CELL COATING SOLUTION (1024-05)

Store at 4°C in the dark upon arrival, The coating solution is stable for 6 months.

D. GI EPITHELIAL CELL THAWING MEDIUM (716T-10)

Store at 4°C in the dark immediately upon arrival. Use within 3 months

Reagent not included in the Total Kit:

GI EPITHELIAL CELL TEER ASSAY EDIUM (716TA-50)

Store at 4°C in the dark immediately upon arrival. Use within 4 weeks.

II. PREPARATION FOR CULTURING

1. Make sure the Class II Biological Safety Cabinet, with HEPA filtered laminar airflow, is in proper working condition.
2. Clean the Biological Safety Cabinet with 70% alcohol to ensure it is sterile.
3. Turn the Biological Safety Cabinet blower on for 10 min. before cell culture work.
4. Make sure all serological pipettes, pipette tips and reagent solutions are sterile.
5. Follow the standard sterilization technique and safety rules:
   a. Do not pipette with mouth.
   b. Always wear gloves and safety glasses when working with human cells even though all the strains have been tested negative for HIV, Hepatitis B and Hepatitis C.
   c. Handle all cell culture work in a sterile hood.

III. CULTURING HGIEpC

A. PREPARING CELL CULTURE WARES FOR CULTURING HGIEpC

1. Take the GI Epithelial Cell Thawing Medium. Decontaminate the bottle with 70% alcohol in a sterile hood.
2. Plan the seeding density in each experiment.
3. Prepare number of wells for culturing HGIEpC by pipetting GI Epithelial Cell Coating Solution per well. (*See tables for cell numbers and volume used*).
4. Incubate coated plate at 37°C for one hour.

B. PREPARING MEDIUM FOR CULTURING HGIEpC

1. Take out GI Epithelial Cell Thawing Medium and transfer 8 ml of the Thawing Medium to a 15 ml tube.
2. Take out the required volume of GI Epithelial Cell Defined Culture Medium according to the Table below and warm up to room temperature.*

***Do not warm Defined Culture Medium to 37°C**

C. THAWING AND PLATING HGIEpC

1. Remove the cryopreserved vial of HGIEpC from the liquid nitrogen storage tank using proper protection for your eyes and hands.
2. Turn the vial cap a quarter turn to release any liquid nitrogen that may be trapped in the threads, then re-

tighten the cap and bury the cryovial in dry ice.

3. Thaw the cells quickly by placing the lower half of the vial in a 37°C water bath and watch the vial closely during the thawing process. This usually takes about 90 sec.
4. Take the vial out of the water bath when only small amount of ice left in the vial. Do not let cells thaw completely.
5. Decontaminate the vial exterior with 70% alcohol in a sterile Biological Safety Cabinet.
6. Remove the vial cap carefully. Do not touch the rim of the cap or the vial.
7. Resuspend the cells in the vial by gently pipetting the cells once with a **pre-wet**, 1 ml aerosol pipette tip set at 950 µl. Do not to pipette vigorously as it might cause foaming.
8. Transfer the cell suspension (1 ml) drop wise to the 15 ml conical tube containing 8 ml GI Epithelial Cell Thawing Medium prepared in Section III B step 1.
9. Add 1 ml of fresh GI Epithelial Cell Thawing Medium to the cryovial for wash. Transfer the wash to the 15 ml tube to a final volume of 10 ml using the same 1 ml aerosol tip in Step 7. Mix by gently inverting the tube couple of times to avoid osmotic shock.
10. Centrifuge cells in the 15 ml conical tube at 200g for 5 minutes. Remove supernatant carefully by aspiration without disturbing the cell pellet. Break the pellet by flickering the tube few times with your finger.
11. Re-suspend HGIEpC by adding 1 ml of GI Epithelial Cell Defined Culture Medium to the cell pellet. Evenly re-suspend the pellet to no visible cell clumps can be seen using a **pre-wet**, 1 ml aerosol pipette tip set at 950 ul.
12. Add 1.5 ml of GI Epithelial Cell Defined Culture Medium to the cell suspension to make final volume of 2.5 ml with cell density of 4 x 10^5/ml.
13. Aspirate GI Epithelial Cell Coating Solution from the wells in the plate prepared in Section IIIA Step 4.
14. Seed HGIEpC suspension to the coated wells as suggested in the reference tables. Rock gently to evenly distribute the cells.
15. Place the plate in a 37°C, 5% CO_2 humidified incubator. For best results, do not disturb the culture for 24 hours after plating.
16. Carefully change to fresh room temperature GIEpC Defined Medium after 24 hours by removing the old medium with pipette tips. **Do not use suction and do not let cells dry up.**
17. **Feed cells every day (within 24 hrs) to prevent involution of the culture. See Table 1 for volumes.**
18. HGIEpC culture will expand in patches in GI Epithelial Cell Defined Culture Medium for up to 5 days before involuting.

TC Well Plate	Volume per Well			Cell Number
	GIEpC Coating Solution	GIEpC Suspension	GIEpC Monolayer in 716DC-50 (Section III C step 14)	Approx. No of HGIEpC required for Normal Culture
96	0.1 ml	200 µl	250 µl	0.8 x 10^5
48	0.15 ml	500 µl	600 µl	2 x 10^5
24	0.25 ml	1 ml	1.2 ml	4 x 10^5

Table: Guide of corresponding cell numbers and working volumes for seeding and maintenance

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