

## General Instructions for Use

### Human Gastrointestinal Epithelial Cells

Instructions apply to Gastric (HGaEpC), Intestinal (HInEpC), Colonic (HCnEpC), Duodenum (HDuEpC), Ileum (HIIEpC), 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-##, 732In-##, 732Cn-##, 732Du-##, 732Je-##)

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

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

Store at -20°C in the dark. The frozen medium is stable for 6 months. Once thawed, keep at 4°C in the dark and use within 4 weeks. Freeze-thaw cycles are not recommended. If necessary, the thawed medium can be aliquoted and refrozen once.

C. GIEpC COATING SOLUTION (025-05)

Store at -20°C. The frozen coating solution is stable for 6 months. Once thawed, keep at 4°C and use within 4 weeks.

D. GIEpC THAWING MEDIUM (716T-20)

Store at -20°C. The frozen medium is stable for 6 months. Once thawed, keep at 4°C and use within 3 months.

#### Reagent not included in the Total Kit:

TEER ASSAY OPTIMIZED GI EPITHELIAL CELL CULTURE MEDIUM (716TA-50)

Store the Culture Medium (716TA-50) at 4°C in the dark immediately upon arrival. Use within 4 weeks of arrival. If not used immediately, store at -20°C upon arrival. The frozen medium is stable for 6 months. Once thawed, keep at 4°C and use within 4 weeks. Re-freeze the culture medium is not recommended.

#### 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 GIEpC Thawing Medium and GIEpC Culture Medium from the refrigerator. Decontaminate the bottle with 70% alcohol in a sterile hood.
2. Plan the seeding density in each experiment.
3. Thaw the GIEpC COATING SOLUTION at 4°C **overnight**. Ensure that the thawing medium is cold enough to avoid gelling.
4. Prepare number of wells of a 48 well plate for culturing HGIEpC by pipetting 150 µl of GIEpC Coating Solution per well. (*See tables for cell numbers and volume used*).
5. Incubate coated plate at room temperature for at least 30 minutes (do not exceed one hour, so adjust your time before taking out the cell vial).

B. PREPARING MEDIUM FOR CULTURING HGIEpC

1. Bring GIEpC Thawing Medium to room temperature. Transfer 8 ml of GIEpC Thawing Medium in a 15 ml tube.
2. Take out the required volume of Culture Medium according to the Table on right side and warm up to room temperature\*.

\* **Do not warm GIEpC 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 GIEpC Thawing Medium prepared in Section III B step 1.
9. Add 1 ml of fresh GIEpC Thawing Medium to the cryovial for wash and add to the 15 ml tube to a final volume of 10 ml. 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. Gently break the pellet by flickering the tube few times with your finger.
11. **Re-suspend HGIEpC at 5 x 10<sup>5</sup>/2.5ml in GIEpC Culture Medium.** Use a **pre-wet**, 1 ml aerosol pipette tip to evenly re-suspend the pellet in 1 ml GIEpC Culture Medium without any visible cell clumps. Then add the rest 1.5 ml medium and gently re-suspend again.
12. Aspirate GIEpC Coating Solution from the wells of the plate prepared in Section III A step 5.
13. Seed HGIEpC suspension to the coated wells as suggested in the reference tables. Rock gently to evenly distribute the cells.
14. Place the plate in a 37°C, 5% CO<sub>2</sub> humidified incubator. For best results, do not disturb the culture for 24 hours after plating.
15. Carefully change to fresh room temperature GIEpC Culture Medium after 24 hours by removing the old medium with 200 ul pipette tips. **Do not use suction and do not let cells dried up.**

16. Change the GIEpC Culture Medium every other day.
17. The HGIEpC monolayer culture should expand in patches in GIEpC Culture Medium for up to 7 days. Cells should be near confluent by day 6.

TC Well Plate	Volume per Well		
	GIEpC Coating Solution	GIEpC Suspension in 716DC-50 (Section III C step 11)	Approx. No of HGIEpC required for Normal Culture
96	0.1 ml	200 µl	0.4 x 10 <sup>5</sup>
48	0.15 ml	500 µl	1 x 10 <sup>5</sup>
24	0.25 ml	1 mL	2 x 10 <sup>5</sup>

**Table 1: Seeding guide of corresponding cell numbers and working volumes for normal seeding**