

General Instructions for Culturing

Human Adipocytes (HAd)

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. PROLIFERATING CELLS (803sD-25, -75, 803hD-25, -75 803sD-6w,-12w,-24w,-48w,-96w; 803hD-6w,-12w,-24w,-48w,-96w)

Examine under a microscope to check if all the cells are attached to the bottom of the flask or wells. If not, notify CAI or your distributor immediately.

- B. ADIPOCYTE DIFFERENTIATION MEDIUM (811D-250)

Store the Differentiation Medium at 4°C in the dark immediately upon arrival.

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. MAINTAINING HAd

Proliferating HAd you received are usually 7-10 days after induction of differentiation.

Do Not Let Cell Dry During The Medium Changes.

- A. PREPARING DIFFERENTIATION MEDIUM

1. Take the Adipocyte Differentiation Medium from the refrigerator. Decontaminate the bottle with 70% alcohol in a sterile hood.
2. Warm the Adipocyte Differentiation Medium to room temperature.

- B. CULTURING HAd

1. Decontaminate the exterior of the flask or plate containing HAd with 70% alcohol.
2. Place the sealed flask or plate in a 37°C, 5% CO₂ humidified incubator for 2 hours as shipped.
3. In a sterile Biological Safety Cabinet, open the cap of the flask or lid of the plate very slowly and carefully.
4. Remove the Medium by aspiration. Add fresh Adipocyte Differentiation Medium according to the following chart.

Culture Format	Surface Area/Well	Volume per well
6 well plate	9.40 cm ²	4 ml
12 well plate	3.83 cm ²	2 ml
24 well plate	1.88 cm ²	1 ml
48 well plate	0.86 cm ²	500 µl
96 well plate	0.32 cm ²	150 µl
T-25 flask	25 cm ²	5 ml
T-75 flask	75 cm ²	15 ml

5. Place the flask or plate in a 37°C, 5% CO₂ humidified incubator with loosened cap to allow gas exchange.
6. Change to fresh Adipocyte Differentiation Medium every 3 days for 15 days.
7. At the end of 15 days, cells are differentiated into HAd with lipid droplets accumulated in the cells.
8. Remove Adipocyte Differentiation Medium and starve the cells in Adipocyte Starving Medium for 1 day prior to assay.

Cell Applications Inc (hereinafter CAI) warrants that its products are manufactured with the utmost care and stringent quality control procedures. However, if you should ever have a problem with the products, we will either replace the products, or in the case we cannot deliver the products, provide you with a refund. Such warranty is applicable only when CAI's cells are used in conjunction with CAI's medium and subculture reagents, and vice versa.