General Instructions for Culturing and Differentiation

HiPSC-Derived Human Brown Fat Preadipocyte Cells (i-HBrPAd) for Mature i-HBrAd

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 (i804-30)

Sold as a pack of 3 vials, 1 x 10E6 per vial. Store the cryovials in a liquid nitrogen storage tank immediately upon arrival.
One vial will seed one well of 6-well plate.

B. THAWING MEDIUM (i811T-30)

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

C. DIFFERENTIATION MEDIUM (i8117DK-15)

Store the DS Basal Medium at 4°C in the dark immediately upon arrival.
Store i8116D-S1, i8117D-S2, and i8117D-S3 at -20°C immediately upon arrival.

D. EXPANSION MEDIUM (i8117EK-10)

Store the ES Basal Medium at 4°C in the dark immediately upon arrival.
Store i8117E-S1, i8117E-S2, at -20°C immediately upon arrival.

E. MATURATION MEDIUM (i8117DK-50)

Store the MS Basal Medium at 4°C in the dark immediately upon arrival.
Store i8117M-S1and i8117M-S2 at -20°C immediately upon arrival.
Store i8117M-S3 at 4°C immediately upon arrival.

F. HiPSC XENO-FREE COATING SOLUTION STOCK (126XF-005)

Store 50 ul of HiPSC Coating Solution Stock of 100X at -20°C immediately after arrival.
Aliquot after first thaw and store the aliquots at -20°C.

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 protective lab gear (lab coat, gloves, safety glasses, etc.) when working with cell cultures.
   c. Handle all cell culture work in a sterile biological safety cabinet.

III. CULTURING i-HBrPAd

A. PREPARING 6-WELL CELL CULTURE PLATE FOR CULTURING i-HBrPAd FROM ONE CRYOVIAL

1. Take out 100x HiPSC Coating Solution Stock from the freezer and briefly centrifuge in microfuge to collect HiPSC Coating Solution Stock.
2. Aliquot 10 uL per vial of HiPSC Coating Solution Stock for single use and store at -20°C.
3. Prepare Working Coating Solution by diluting HiPSC Coating Solution Stock 1:100 in sterile PBS: 10 ul to 1 ml in PBS. Working Coating Solution is only stable for 2 weeks at 4°C.
5. Prepare one well of 6-well plate by pipetting 1 ml of HiPSC Working Coating Solution to coat a well.
6. Incubate coated 6-well plate at 37ºC for one hour.
7. Do not aspirate HiPSC Working Coating Solution from the 6-well plate until just before cells are ready for seeding.
   *Consult Appendix for seeding thawed i-HBrPAd product vial in different culture format.

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.

B. PREPARING DIFFERENTIATION MEDIUM FOR CULTURING i-HBrPAd

1. Take the DS Basal Differentiation Medium from the refrigerator. Decontaminate the bottle with 70% alcohol in a sterile biological safety cabinet.
2. Thaw i-HBrPAd Differentiation Supplements i8117D-S1, i8117D-S2 and i8117D-S3. Add all three Differentiation Supplements, to the DS Basal Medium and mix gently.
3. Store the Complete Medium at 4°C. It should be used within one week following preparation.

IV. THAWING, PLATING, AND DIFFERENTIATING i-HBrPAd

Plating the i-HBrPAd is a selective process. Though viable, roughly 80% of the cells will NOT adhere to the plate. This is expected and non-adherent cells are not target cells. Plated i-HBrPAd are expected to proliferate robustly.

1. For each cryovial to be thawed, remove 9 mL of Thawing Medium from 4°C storage and transfer to a 15 mL conical tube. Bring Thawing Medium to room temperature.
2. Remove the cryopreserved vial of i-HBrPAd from the liquid nitrogen storage tank using proper protection for your eyes and hands.
3. Turn the vial cap a quarter turn to release any liquid nitrogen that may be trapped in the threads, then re-tighten the cap.
4. 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.
5. Take the vial out of the water bath when only small amount of ice left in the vial. Do not let cells thaw completely.
6. Decontaminate the vial exterior with 70% alcohol in a sterile biological safety cabinet.
7. Remove the vial cap carefully. Do not touch the rim of the cap or the vial.
8. Resuspend the cells in the vial by gently pipetting the cells two times with pre-wetted, 1 mL aerosol tip set at 950 uL. Be careful not to pipette vigorously and cause foaming.
9. Pipette the cell suspension (1ml) from the vial to a 15 ml sterile conical tube containing 9 ml of Thawing Medium.
10. Centrifuge the conical tube at 220 x g for 5 minutes to pellet the cells.
11. Aspirate the supernatant from the tube without disturbing the cell pellet.
12. Flick the tip of the conical tube with your finger to loosen the cell pellet.
13. Resuspend the cells in 2 ml of Differentiation Medium by gently pipetting the cells to break up the cell pellet.
14. Aspirate HiPSC Working Coating Solution from the 6-well plate and immediately transfer the cell suspension from the tube into the 6-well plate.
15. Close lid and rock gently to evenly distribute the cells.
16. Place the 6-well plate in a 37ºC, 5% CO_2 humidified incubator.
17. Feed with fresh Differentiation Medium after two days.
18. Total time in Differentiation Medium: four days.

IV. EXPANDING i-HBrPAd

1. Take the ES Basal Medium from the refrigerator. Decontaminate the bottle with 70% alcohol in a sterile biological safety cabinet.
2. Thaw i-HBrPAd Expansion Supplements i8117E-S1 and i8117E-S2. Add both Expansion Supplements to the ES Basal Medium and mix gently.
3. Store the Complete Medium at 4°C. It should be used within one week following preparation.
4. Remove the Differentiation Medium and feed the adherent cells once with room-temperature Expansion Media.
5. Total time in Expansion Medium: two days

V. MATURING i-HBrPAd

1. After two days in Expansion Media, take the MS Basal Medium from the refrigerator. Decontaminate the bottle with 70% alcohol in a sterile biological safety cabinet.
2. Thaw i-HBrPAd Maturation Supplements i8117M-S1 and i8117M-S2. Add all three Maturation Supplements (including i8117M-S3) to the MS Basal Medium and mix gently.
3. Store the Complete Medium at 4°C. It should be used within three weeks following preparation.
4. Remove the Expansion Medium and feed the adherent cells with room temperature Maturation Medium. Change with fresh Maturation Medium every other day. i-HBrPAd cells are considered mature after two weeks culture in Maturation Medium.
5. i-HBrPAd cells are not capable of passaging but will divide to fill the well. Lipid droplets should appear within 48 hours in Maturation Medium and continue to become more prominent throughout the culture for up to three weeks upon application of Maturation Medium.

APPENDIX

Seeding Guide of Cryopreserved i-HBrPAd Product Vial after Thawing.

TC Vessel	Number of wells per cryovial	Number of cells per well	Volume per Well	
			Working Coating Solution	i-HBrPAd Medium
12-Well Plate	3 wells	3 x 10e5	0.5 ml	1 ml
6-Well Plate	1 well	1 x 10e6	1 ml	2 ml
T-25 Flask	Not recommended	n/a	2 ml	5 ml

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