NEUROMICS

Primary Mouse Whole Heart

Catalog Number: PC35133 **Components: ● E18 or P2 whole heart tissue - >1 million viable cells**
● 12 mls Culture Media- 12 ml of NbCardio: Cardiomyocyte growth media

Description: Fresh intact heart tissue from an E18 or P2 C57/Bl6 mouse and media provided to initiate a culture of cardiomyocytes. This tissue allows you to create a cardiomyocyte culture that retains its physiological functions, including beating.

Shipping/Storage: Use tissue immediately for highest cell yield; however, tissue can be stored for one week at 4-8°C.

Culture Protocol

Preparation (Room Temperature in a Sterile Hood

1. Prepare cell dissociation solution by adding 3 ml of HE direction into the 6 mg vial of Papain (2 mg/ml). Recap the vial, gently mix, and place in 30°C water bath for 10 min to dissolve. Remove from the water bath and allow to come to room temperature
2. Add 80µl of Trypan Blue to a 0.5 ml centrifuge tube for Step 9.

Cell Dispersal (Room Temperature in a Sterile Hood)

1. With the Pasteur pipette, remove the tissue with minimal Cardio Transport / Maintenance media and place in a 1.5 centrifuge tube with 1 ml of cell dissociation solution. Return excess Cardio Transport / Maintenance media to vial.
2. Seal the tube with cell dissociation solution and incubate in a 37°C water bath/incubator (optional: 100-150 RPM if shaker available) for 30 minutes. Gently swirl every 5 minutes.
3. With a pipetman and sterile tip carefully remove the cell dissociation solution and wash the tissue with 1 ml of HE media
4. With the Pasteur pipette, draw the tissue with minimal HE medium into the pipette and immediately dispense contents into the Cardio Transport/Maintenance tube taking care to avoid air bubbles. Triturate heart tissue for ~1 min (90% tissue dispersal)
5. Transfer entire supernatant containing dispersed cells and tissue debris to the sterile 40 µm cell strainer (pre-wet with 300 µl of HE media) and pass through the mesh into a sterile 50 ml centrifuge tube. Remove the strainer and place cell suspension in a sterile 15 ml centrifuge tube.
6. Spin 2000 rmp (~600 x G), 5 min. Discard supernatant leaving ~50 µl of Cardio Transport / Maintenance media containing the pellet.
7. Aliquot 20 µl of cell solution into the 0.5 ml tube containing 80 µl of Trypan Blue (1:5 dilution)
8. Count cells using a hemacytometer (calculate cells/ml and viability)

Cell Plating (Room Temperature in a Sterile Hood)

1. Dilute cells with cardiac cell culture media (0.2 ml/cm^2) and plate at 75,000 cells/cm^2 or desired concentration on 0.1% gelatin coated slips/flasks/wells.
2. Incubate 37°C, 5% CO_2, 9% O_2, 95% humidity (or ambient O_2)
3. After 24 hours, cardiomyocytes display spontaneous contracting and syncytial contracting at 120 hours
4. Change the medium with fresh, 37°C, CO_2 equilibrated cardiac cell media or media of choice every 2 days
    a. Around day 5 when the cells are confluent and beating in rhythm, we suggest switching from cardiomyocyte growth media to cardiomyocyte maintenance media.

Viability Assay

1. Rinse cells twice with 37°C HBSS (0.2 ml/cm^2 of substrate).
2. Prepare dye mix from an acetone stock of 15 mg/ml fluorescein diacetate and an aqueous stock of 4.6 mg/ml propidium iodide, dilute 15 µl of each into 1.5 ml HBSS (1:100 dilution).
3. Add 20 µl of dye mix from step 2 to every 0.2 ml of HBSS added in step 1 (1:10 dilution).
4. After ~1 min count live cells using blue excitation appropriate for fluorescein fluorescence (green cells). Count dead cells with green excitation for propidium iodide fluorescence (small red nuclei).
5. Viability = (green cells/unit area)/(total cells plated/unit area) or Survival = green cells/(green + red cells)

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