NEUROMICS

Primary Mouse Whole Heart Dissociated Cells

Catalog Number: PC35136 **Components: ● Dissociated heart of E18 or P2 Mouse - >1 million viable cells**
● 12 mls Culture Media- 12 ml of NbCardio: Cardiomyocyte growth media

Description: Fresh dissociated heart cells from E18 or P2 C57/Bl6 Mouse and media provided to initiate a culture of cardiomyocytes.

Shipping/Storage: Use dissociated cardiomyocytes immediately for highest cell yield; however, tissue can be stored for one week at 4-8°C. Dissociated heart is provided in 2 ml of Cardio Transport /Maintenance media.

Culture Protocol

Preparation (Room Temperature in a Sterile Hood

1. Prepare substrate by coating slips or well plates with Gelatin (coating is 0.1% in filter sterilized DI water). Allow to sit for at least 3 hours to overnight at room temperature, wash once with DI water and allow to dry completely.
2. 2. Fire polish the tip of a sterile 9" silanized pasteur pipette to an opening of ~0.5 mm
3. 3. Aliquot 80 µl of Trypan Blue (Sigma: T8154) into a 0.5 ml tube for Step 6.

Cell Dispersal (Room Temperature in a Sterile Hood)

1. Allow 12 mL of NbCardio to come to room temperature.
2. 2. Warm the 2 mL tube of dissociated cardiomyocytes in a 30ºC water bath for 1 min.
3. 3. With the silanized pasteur pipette, transfer the entire tube's contents to a sterile 15 ml centrifuge tube and triturate no more than 5 times. There should be no visible aggregation of cells in the media.
4. 4. Spin 2000 rpm (~650 x G), 5 min. Discard supernatant leaving ~50 µl of Cardio Transport/ Maintenance media containing the pellet.
5. 5. Disperse the pellet of cells (flick the bottom of the tube with a finger) and resuspend pellet in 1 ml NbCardio
6. 6. Aliquot 20 µl of cell solution into the 0.5 ml tube containing 80 µl of Trypan Blue (1:5 dilution).
7. 7. Count cells using a hemacytometer (calculate cells/ml).

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)

Neuromics • 5325 West 74th Street, Suite 8 • Edina, MN 55439
phone 866-350-1500 • fax 612-677-3976 • e-mail pshuster@neuromics.com