Miltenyi Biotec

Dissociation of PSC-derived cardiomyocytes using the Multi Tissue Dissociation Kit 3

Contents

1. Description

1.1 Background information

Single-cell suspensions are a prerequisite for many experiments, for example to achieve the highest possible purity and recovery during cell separations with MACS® Technology.

This protocol has been developed to obtain single cells from pluripotent stem cell (PSC)–derived cardiomyocytes using the Multi Tissue Dissociation Kit 3.

1.2 Reagent and instrument requirements

- Multi Tissue Dissociation Kit 3 (# 130-110-204)
- Cell culture medium with 20% fetal bovine serum (FBS)
- Phosphate-buffered saline (PBS), pH 7.4
- MACS SmartStrainers (70 µm) (# 130-098-462)

2. Protocol for the dissociation of PSC-derived cardiomyocytes

▲ For cell culture experiments subsequent to tissue dissociation, all steps should be performed under sterile conditions.

▲ The dissociation protocol have been optimized for the use with 12-well or 6-well plates.

1. Remove cell culture supernatant from the cultured cells.
2. Wash with the appropriate amount of PBS:
   12-well plate: 3×1 mL 6-well plate: 3×2 mL
3. Prepare enzyme mix by adding Enzyme T to Buffer X of the Multi Tissue Dissociation Kit 3 in a ratio of 1:10, for example, add 50 µL of Enzyme T to 450 µL of Buffer X.
4. Add the appropriate amount of the enzyme mix per well:
   12-well plate: 400 µL 6-well plate: 1 mL
5. Incubate sample for 10 minutes at 37 °C.
6. Add the appropriate amount of cell culture medium with 20% FBS:
   12-well plate: 600 µL 6-well plate: 1 mL
7. Detach cells from the dish very gently by pipetting 3× up and down using a 1 mL pipette.
   ▲ **Note:** For late stage differentiation it might be needed to pipette more often.
8. Apply the cells to a MACS SmartStrainer (70 µm) placed on a 50 mL tube.
9. Wash each well with the appropriate amount of cell culture medium with 20% FBS and also apply to the MACS SmartStrainer (70 µm):
   12-well plate: 1 mL 6-well plate: 2 mL
10. Wash MACS SmartStrainer (70 µm) with the appropriate amount of cell culture medium with 20% FBS:
    12-well plate: 1 mL 6-well plate: 2 mL
11. Determine the cell number and continue with further applications.

All protocols and data sheets are available at **www.miltenyibiotec.com.**

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