

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

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1. Description

This product is for research use only.

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
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3. Prepare enzyme mix by adding Enzyme T to Buffer X of the Multi Tissue Dissociation Kit 3 in a ratio of 1:33, for example, add 16.7 µL of Enzyme T to 483.3 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
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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
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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
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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
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11. Determine the cell number and continue with further applications.

Refer to www.miltenyibiotec.com for all data sheets and protocols. Miltenyi Biotec provides technical support worldwide. Visit www.miltenyibiotec.com for local Miltenyi Biotec Technical Support contact information.

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