



Application note

Dissociation of embryoid bodies

Protocol for the dissociation of embryoid bodies using the gentleMACS™ Dissociator, followed by the isolation of putative SSEA1⁺ germ cells

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Introduction

Protocols for the differentiation of ES and iPS cells frequently involve the formation of embryoid bodies (EBs) as an initial step in the generation of different cell types through spontaneous differentiation. As EBs contain a heterogeneous cell population, specific isolation of target cells from EBs is often a necessary step to promote differentiation into specific lineages. Complete dissociation of EBs into viable single cells is a prerequisite for reliable cell separation and cell analysis. The gentleMACS™ Dissociator helps streamline and standardize this process for reproducible results. This protocol exemplifies the use of the instrument for the dissociation of human EBs followed by indirect immunomagnetic isolation of SSEA-1–positive presumptive mesodermal cells using CD15 (SSEA1)-FITC antibody followed by Anti-FITC MicroBeads.

Materials

Instruments

gentleMACS™ Dissociator (Miltenyi Biotec, # 130-093-235)
MACSmix™ Tube Rotator (Miltenyi Biotec, # 130-090-753)

Disposables

gentleMACS C Tubes (Miltenyi Biotec, # 130-093-237)
StemPro® EZ Passage™ Tools (Invitrogen Life Technologies)

Cell separation and analysis reagents

Anti-FITC MicroBeads (Miltenyi Biotec, # 130-048-701);
CD15 (SSEA1)-FITC, human, antibody (Miltenyi Biotec,
130-081-101); CD117 (A3C6E2)-APC, human, antibody
(Miltenyi Biotec, # 130-091-733)

Cell culture equipment

DMEM/F12 medium with HEPES and GlutaMAX™,
Ca²⁺/Mg²⁺-free PBS, and Collagenase type IV (Invitrogen
Life Technologies); Fetal calf serum (FCS) (Hyclone);
Recombinant human BMP4, Accutase (Stemgent)

Protocol

Cell culture

1. Incubate undifferentiated human embryonic stem cells (WA01 or WA09) grown on mouse embryonic fibroblasts (MEFs) in 2 mg/mL of collagenase type IV at 37 °C for 5 minutes.
2. Aspirate enzyme solution and rinse twice with 2 mL of DMEM/F12 medium.
3. Add 1 mL of DMEM/F12 with 20% FCS. Cross-cut colonies with a StemPro EZ Passage Tool and dislodge cut colonies by gentle pipetting.
4. Transfer colonies in suspension to a 15 mL conical vial.
5. Allow colonies to settle for 5 minutes and aspirate MEFs in suspension.
6. Resuspend pellet in 5 mL of DMEM/F12 supplemented with 20% FCS and 8 ng/mL BMP4 and plate in a low-adhesion 6-well plate.
7. Change the medium every other day with DMEM/F12, supplemented with 20% FCS and 8 ng/mL BMP4.
8. On day 8, collect EBs in suspension and centrifuge for 10 minutes at 200×g.
9. Carefully aspirate media, as cystic EBs may float.



EB dissociation

Note: Alternatively, the new Embryoid Body Dissociation Kit can be used.

1. Resuspend EB pellet in 3 mL of 2 mg/mL collagenase Type IV and transfer to gentleMACS™ C Tube.
2. Place C Tube into MACSmix™ Tube Rotator in a 37 °C incubator on constant slow rotation setting for 10 minutes.
3. Transfer C Tube into gentleMACS™ Dissociator, run gentleMACS Program "h_tumor_01".
4. Add 7 mL of Ca²⁺/Mg²⁺-free PBS, transfer into a 15 mL conical vial and centrifuge at 200×g for 5 minutes.
5. Aspirate supernatant and resuspend in 3 mL of accutase and transfer into C Tube.
6. Incubate at 37 °C for 1 minute without agitation.
7. Transfer to gentleMACS Dissociator, run gentleMACS Program "h_tumor_02".
8. Add 7 mL of DMEM/F12, supplemented with 20% FCS, w/o growth factors; transfer to a 15 mL conical vial and centrifuge at 200×g for 5 minutes.

Cell isolation and analysis

1. Use single-cell suspension for subsequent cell isolation and analyses.
2. Follow instructions for indirect MACS® Separation using Miltenyi Biotec's CD15 (SSEA1)-FITC antibody followed by Anti-FITC MicroBeads.

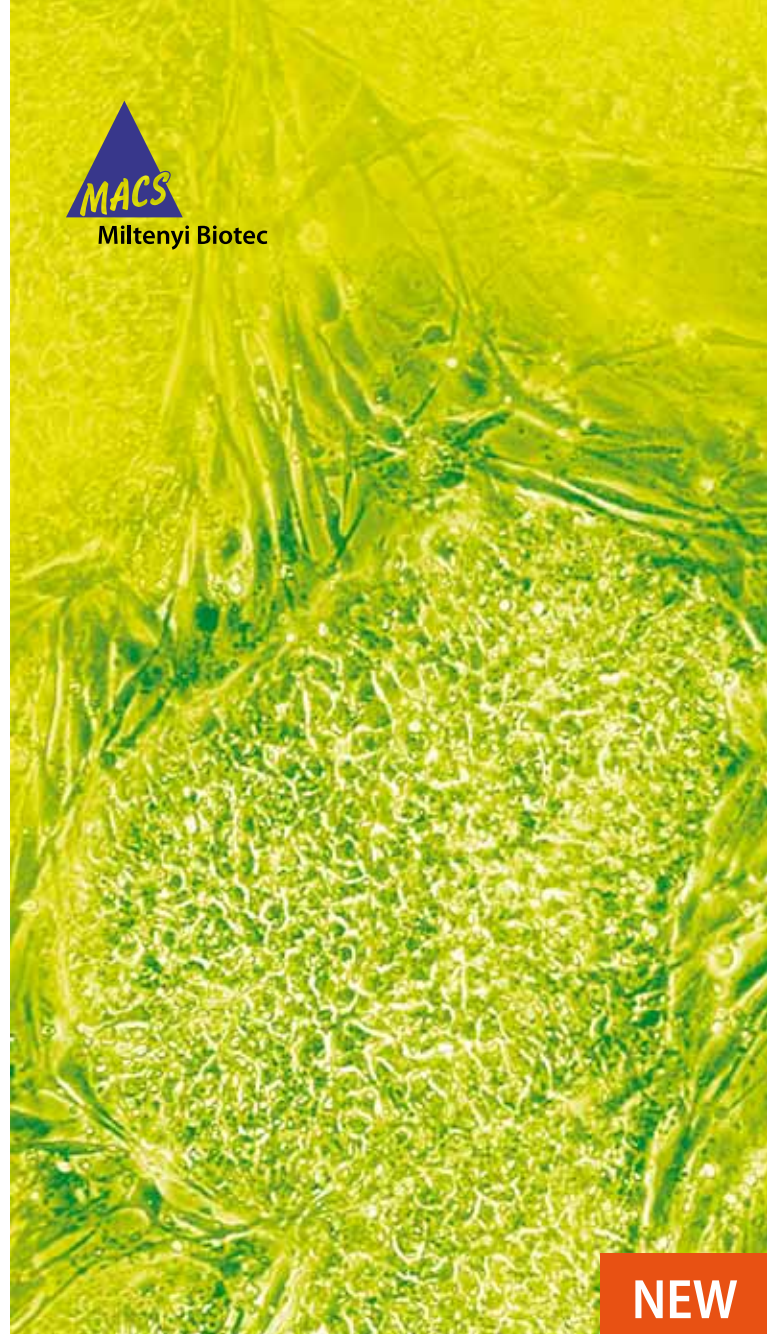
Note: Alternatively, Anti-SSEA-1 (CD15) MicroBeads can be used for direct magnetic labeling.

3. Confirm expression of secondary markers for putative primordial germ cells in the target cell population by live staining for extracellular markers such as c-KIT (CD117) using anti-human CD117 (A3C6E2)-APC antibody following Miltenyi Biotec's product insert protocol.



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NEW

Improve your dissociation of embryoid bodies

Embryoid Body Dissociation Kit, human and mouse (# 130-096-348):

- Effective dissociation of embryoid bodies
- Excellent reproducibility, high viability rates
- Closed, sterile system
- Easy-to-use enzyme mix, optimized for use with the gentleMACS™ Dissociator

Contact us for more information:
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► macs-stemcells.com