Quick-Tissue™
Mesoderm Booster

Catalog Number: EXGS-QTMB

User Manual

This product (EXGS-QTMB) contains 3 tubes for use with a total of 6 wells of a 24-well plate or two full 4-well plates.

Table of Contents:

I. Introduction 2
II. Contents 2
III. Pre-Protocol Preparation 2
IV. Basic Protocol 3
V. Detailed Protocol Examples 4
VI. Appendix A: Reference Pictures and Figures 10
VII. Appendix B: Troubleshooting 10
VIII. Appendix C: Literature References 11
I. Introduction

Thank you for purchasing Quick-Tissue™ Mesoderm Booster. This product allows users to accelerate the differentiation of human pluripotent stem cells (hPSCs), including embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs), toward the mesodermal lineage when used in combination with the user’s own differentiation induction method.

NOTE: EXPERIMENT SCALE

- This product contains enough reagents to boost differentiation of hPSCs in 6 wells of a 24-well plate. For other well sizes, multiply the protocol’s recipe by the ratio between well sizes.
- For first time users of this product, we recommend optimizing the timing and frequency of application of Mesoderm Booster for best integration into the user’s differentiation induction method.

II. Kit Contents

Upon receipt of this kit, immediately store all reagents at their proper storage temperatures as described in the table below. All reagents are shipped on dry ice.

List of Components

<table>
<thead>
<tr>
<th>Reagents</th>
<th>Amount</th>
<th>Storage Conditions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mesoderm Booster</td>
<td>55 µl x 3</td>
<td>-80 °C</td>
</tr>
</tbody>
</table>

* This kit contains Viromer® (Lipocalyx GmbH).

III. Pre-Protocol Preparation

NOTE: SOURCE hPSC CULTURE CONDITIONS

- The recipe in the Quick-Tissue™ Mesoderm Booster Kit protocol assumes that the undifferentiated hPSCs (starting materials) are cultured in wells of a 24-well plate.

NOTE: CELL DENSITY

- In this protocol, we recommend aiming for 40-60% confluency with 20-50 cells per colony on the day when Mesoderm Booster is used.
  - Our data indicate that cell counts ranging from 0.6 - 1.2 x 10^5 cells per well one day ahead of Mesoderm Booster treatment.
  - Cell count may vary based on cell health, the method, and instrument used for cell counting.

- We do not recommend additional freeze-thaw cycles of this reagent.
IV. Basic Protocol

Mesoderm Booster accelerates hPSC differentiation by strongly suppressing the pluripotency maintenance program in hPSCs. It is intended to be used at the beginning of PSC differentiation. Typically, the booster is added immediately after hPSCs are put into differentiation conditions. However, users may want to add the booster at various timings before or after the culture conditions of the hPSCs are switched from pluripotency maintenance to differentiation.

Regardless of differentiation induction methods, cultures with 40-60% confluency should be treated with Mesoderm Booster by the following basic steps:

1. Thaw Mesoderm Booster on ice for 20-30 minutes.
2. Pipet out old medium from each hPSC culture using a P1000 pipettor and add 250 μl medium to it.
3. Incubate Mesoderm Booster at room temperature for 5 minutes. Simply tap the side of the vial or gently pipet up and down to mix it well.
4. Add 25 μl Mesoderm Booster to each culture by adding Mesoderm Booster dropwise with one hand and simultaneously gently shaking the plate using the other hand.
5. Swirl the plate for 15 seconds to make sure that Mesoderm Booster is evenly distributed in the cultures.
6. Incubate the cultures at 37°C, 5% CO₂ for at least 3 hours.
7. The cultures are ready for the subsequent differentiation induction procedure.

These steps can be performed (A) before, (B) at the beginning of, or (C) after differentiation induction as indicated in the outline below. Differentiation induction (*) can be carried out by the following methods: (1) the use of specialized culture media, (2) delivery of RNA (i.e., transfection), and (3) transcriptional activation of an inducible gene expression cassette stably integrated in the genome of hPSCs. If necessary, adjust the volume of culture medium and Mesoderm Booster proportionally. It is possible to leave the cultures with the medium including Mesoderm Booster overnight. However, depending on culture conditions, the hPSCs may exhibit susceptibility to Mesoderm Booster when it is left in the culture overnight.

A. Before Differentiation Induction

- - - - -

Confluence should be 40-60%

Differentiation Day 1

B. At the Beginning of Differentiation Induction

- - - - -

Confluence should be 40-60%

Differentiation Day 1

C. After Differentiation Induction

- - - - -

Confluence should be 40-60%

Differentiation Day 1
V. Detailed Protocol Examples

Example applications of Mesoderm Booster to currently existing differentiation induction methods are described below.

(1) For a medium-based differentiation method
The following protocol is designed for those who maintain standard hPSCs and induce their differentiation by replacing a culture medium for undifferentiated conditions with a medium for differentiation conditions a few days after plating.

![Diagram of differentiation induction process]

**NOTE: CELL CULTURE TIPS**
- It is possible to leave the cultures with the medium including Mesoderm Booster overnight. However, the hPSCs may exhibit susceptibility to Mesoderm Booster when it is left in the culture overnight.

**Treatment option A: before differentiation induction**

**Day 0**
1. Make sure that the cultures have 40-60% confluency with 20-50 cells per colony (possibly on the cell plating day or a few days after cell plating). If not, wait another day or start with a fewer number of cells plated.
2. Thaw Mesoderm Booster on ice for 20-30 minutes.
3. Pipet out old medium from each well using a P1000 pipettor and add 250 μl maintenance medium such as StemFit®, mTeSR1®, E8®, etc. to it.
4. Incubate Mesoderm Booster at room temperature for 5 minutes. Gently pipet up and down to mix it well.
5. Add 25 μl Mesoderm Booster to each culture by adding Mesoderm Booster dropwise with one hand and simultaneously gently shaking the plate using the other hand.
6. Swirl the plate for 15 seconds to make sure that Mesoderm Booster is evenly distributed in each culture.
7. Incubate the cultures at 37°C, 5% CO₂ for at least 3 hours.
8. Pipet out the old medium with Mesoderm Booster from each culture using a P1000 pipettor and add 500 μl maintenance medium to it.
9. Incubate the cultures at 37°C, 5% CO₂ overnight.
10. Start user’s differentiation induction method next day.

**Treatment option B: at the time of differentiation induction**

**Day 1**
1. Make sure that the cultures have 40-60% confluency with 20-50 cells per colony. If not, wait another day or start with a fewer number of cells plated.
2. Thaw Mesoderm Booster on ice for 20-30 minutes.
3. Pipet out old medium from each culture using a P1000 pipettor and add 250 μl differentiation medium to it.
4. Incubate Mesoderm Booster at room temperature for 5 minutes. Gently pipet up and down to mix it well.
5. Add 25 μl Mesoderm Booster to each culture by adding Mesoderm Booster dropwise with one hand and simultaneously gently shaking the plate using the other hand.
6. Swirl the plate for 15 seconds to make sure that Mesoderm Booster is evenly distributed in each culture.
7. Incubate the cultures at 37°C, 5% CO₂ for at least 3 hours.
8. Pipet out the old medium with Mesoderm Booster from each culture using a P1000 pipettor and add 500 μl differentiation medium to it. Incubate the cultures at 37°C, 5% CO₂ and follow user’s differentiation induction method.
9. Incubate the cultures at 37°C, 5% CO₂ and follow user’s differentiation induction method.

Treatment option C: consecutive treatments on day 0 and day 1

Day 0
1. Make sure that the cultures have 40-60% confluency with 20-50 cells per colony. If not, wait another day or start with a fewer number of cells plated.
2. Thaw Mesoderm Booster on ice for 20-30 minutes.
3. Pipet out old medium from each well using a P1000 pipettor and add 250 μl maintenance medium such as StemFit®, mTeSR1®, E8®, etc. to it.
4. Incubate Mesoderm Booster at room temperature for 5 minutes. Gently pipet up and down to mix it well.
5. Add 25 μl Mesoderm Booster to each culture by adding Mesoderm Booster dropwise with one hand and simultaneously gently shaking the plate using the other hand.
6. Swirl the plate for 15 seconds to make sure that Mesoderm Booster is evenly distributed in each culture.
7. Incubate the cultures at 37°C, 5% CO₂ for at least 3 hours.
8. Pipet out the old medium with Mesoderm Booster from each culture using a P1000 pipettor and add 500 μl maintenance medium to it.
9. Incubate the cultures at 37°C, 5% CO₂ overnight.

Day 1
1. Thaw Mesoderm Booster on ice for 20-30 minutes.
2. Pipet out the old medium with Mesoderm Booster from each culture using a P1000 pipettor and add 250 μl differentiation medium to it.
3. Incubate Mesoderm Booster at room temperature for 5 minutes. Gently pipet up and down to mix it well.
4. Add 25 μl Mesoderm Booster to each culture by adding Mesoderm Booster dropwise with one hand and simultaneously gently shaking the plate using the other hand.
5. Swirl the plate for 15 seconds to make sure that Mesoderm Booster is evenly distributed in each culture.
6. Incubate the cultures at 37°C, 5% CO₂ for at least 3 hours.
7. Pipet out the old medium with Mesoderm Booster from each culture using a P1000 pipettor and add 500 μl differentiation medium to it.
8. Incubate the cultures at 37°C, 5% CO₂ and follow user’s differentiation induction method.
(2) For a transfection-based method

The following protocol is designed for those who maintain standard hPSCs and induce their differentiation by transfection of RNA encoding a transgene(s) without selection of cells that stably express the transgene.

Day 0 - Plating

1. Plate 0.6-1.2 \times 10^5 hPSCs per well. Please make sure that cells are evenly distributed in the wells by gently rocking the entire plate several times.
2. Incubate the cultures at 37°C, 5% CO\textsubscript{2} overnight.

NOTE: CELL CULTURE TIPS

- hPSCs will unevenly distribute in culture when extensive swirling or shaking is applied while single hPSCs are settling down after plating. Distribution of hPSCs in culture affects the efficacy of Mesoderm Booster.
- It is possible to leave the cultures with the medium including Mesoderm Booster overnight. However, the hPSCs may exhibit susceptibility to Mesoderm Booster when it is left in the culture overnight.

Treatment option A: before transfection

Day 1

1. Thaw Mesoderm Booster on ice for 20-30 minutes.
2. Pipet out old medium from each well using a P1000 pipettor and add 250 μl medium used for the following transfection to each culture.
3. Incubate Mesoderm Booster at room temperature for 5 minutes. Gently pipet up and down to mix it well.
4. Add 25 μl Mesoderm Booster to each culture by adding Mesoderm Booster dropwise with one hand and simultaneously gently shaking the plate using the other hand.
5. Swirl the plate for 15 seconds to make sure that Mesoderm Booster is evenly distributed in each culture.
6. Incubate the cultures at 37°C, 5% CO\textsubscript{2} for at least 3 hours.
7. Add more fresh medium to adjust the culture volume for transfection (i.e., there is no need to remove Mesoderm Booster for the following transfection) to each culture. The cultures are ready for transfection.
8. Incubate the cultures at 37°C, 5% CO\textsubscript{2} and follow user’s transfection and differentiation induction method.

Treatment option B: at the time of transfection

Day 1

1. Thaw Mesoderm Booster on ice for 20-30 minutes.
2. Pipet out old medium from each well using a P1000 pipettor and add 500 μl medium used for the following transfection to each culture.
3. Prepare user’s transfection reagent.
4. Incubate Mesoderm Booster at room temperature for 5 minutes. Gently pipet up and down to mix it well.
5. Add 50 μl Mesoderm Booster to each culture by adding Mesoderm Booster dropwise with one hand and simultaneously gently shaking the plate using the other hand. Immediately after the Mesoderm Booster treatment, add user’s transfection reagent in the cultures.
6. Swirl the plate for 15 seconds to make sure that Mesoderm Booster is evenly distributed in each well.
7. Immediately after the Mesoderm Booster treatment, add user’s transfection reagent to each well.
8. Incubate the cultures at 37°C, 5% CO₂ for 3 hours.
9. Pipet out the old medium with Mesoderm Booster and the transfection reagent from each culture using a P1000 pipettor and add 500 μl medium.
10. Incubate the cultures at 37°C, 5% CO₂ and follow user’s differentiation induction method.

Treatment option C: after transfection

Day 1
1. Follow user’s method and perform transfection.
2. Incubate the cultures at 37°C, 5% CO₂ for at least 3 hours.
3. Thaw Mesoderm Booster on ice for 20-30 minutes.
4. Pipet out old medium from each culture using a P1000 pipettor and add 250 μl medium used for the following transfection to each well.
5. Incubate Mesoderm Booster at room temperature for 5 minutes. Gently pipet up and down to mix it well.
6. Add 25 μl Mesoderm Booster to each culture by adding Mesoderm Booster dropwise with one hand and simultaneously gently shaking the plate using the other hand.
7. Swirl the plate for 15 seconds to make sure that Mesoderm Booster is evenly distributed in each culture.
8. Incubate the cultures at 37°C, 5% CO₂ for at least 3 hours.
9. Pipet out the old medium with Mesoderm Booster from each culture using a P1000 pipettor and add 500 μl differentiation medium to it.
10. Incubate the cultures at 37°C, 5% CO₂ and follow user’s differentiation induction method.
(3) For a gene induction-based method

The following protocol is designed for those who maintain genetically engineered hPSCs and induce their differentiation by the addition of an inducer such as doxycycline and tamoxifen that activates forced expression of an inducible transgene integrated into the cells’ genome.

![Diagram showing the process of gene induction]

**Day 0 - Plating**

1. Plate 0.6-1.2 x 10^5 hPSCs per well. Please make sure that cells are evenly distributed in the wells by gently rocking the entire plate several times.
2. Incubate the cultures at 37°C, 5% CO₂ overnight.

**NOTE: CELL CULTURE TIPS**

- hPSCs will unevenly distribute in culture when extensive swirling or shaking is applied while single hPSCs are settling down after plating. Distribution of hPSCs in culture affects the efficacy of Mesoderm Booster.
- It is possible to leave the cultures with the medium including Mesoderm Booster overnight. However, the hPSCs may exhibit susceptibility to Mesoderm Booster when it is left in the culture overnight.

**Treatment option A: before induction**

**Day 1**

1. Thaw Mesoderm Booster on ice for 20-30 minutes.
2. Pipet out old medium from each culture using a P1000 pipettor and add 250 μl medium used for the gene induction without an inducer (e.g., doxycycline) to it.
3. Incubate Mesoderm Booster at room temperature for 5 minutes. Gently pipet up and down to mix it well.
4. Add 25 μl Mesoderm Booster to each culture by adding Mesoderm Booster dropwise with one hand and simultaneously gently shaking the plate using the other hand.
5. Swirl the plate for 15 seconds to make sure that Mesoderm Booster is evenly distributed in each culture.
6. Incubate the cultures at 37°C, 5% CO₂ for at least 3 hours.
7. Pipet out the old medium from each culture using a P1000 pipettor and add 500 μl medium used for the gene induction with an inducer (e.g., doxycycline) to it.
8. Incubate the cultures at 37°C, 5% CO₂ and follow user’s differentiation induction method.

**Treatment option B: at the time of induction**

**Day 1**

1. Thaw Mesoderm Booster on ice for 20-30 minutes.
2. Pipet out old medium from each culture using a P1000 pipettor and add 250 μl medium used for the gene induction with an inducer (e.g., doxycycline) to it.
3. Incubate Mesoderm Booster at room temperature for 5 minutes. Gently pipet up and down to mix it well.
4. Add 25 μl Mesoderm Booster to each culture by adding Mesoderm Booster dropwise with one hand and simultaneously gently shaking the plate using the other hand.
5. Swirl the plate for 15 seconds to make sure that Mesoderm Booster is evenly distributed in each culture.
6. Incubate the cultures at 37°C, 5% CO₂ for at least 3 hours.
7. Pipet out the old medium from each culture using a P1000 pipettor and add 500 μl medium used for the gene induction with an inducer (e.g., doxycycline) to it.
8. Incubate the cultures at 37°C, 5% CO₂ and follow user’s differentiation induction method.

**Treatment option C: after induction**

**Day 1**
1. Thaw Mesoderm Booster on ice for 20-30 minutes.
2. Pipet out old medium from each culture using a P1000 pipettor and add 250 μl medium used for the gene induction with an inducer (e.g., doxycycline) to it.
3. Incubate the cultures at 37°C, 5% CO₂ for at least 3 hours.
4. Add 25 μl Mesoderm Booster to each culture by adding Mesoderm Booster dropwise with one hand and simultaneously gently shaking the plate using the other hand.
5. Swirl the plate for 15 seconds to make sure that Mesoderm Booster is evenly distributed in each culture.
6. Incubate the cultures at 37°C, 5% CO₂ for at least 3 hours.
7. Pipet out the old medium from each culture using a P1000 pipettor and add 500 μl medium used for the gene induction with an inducer (e.g., doxycycline) to it.
8. Incubate the cultures at 37°C, 5% CO₂ and follow user’s differentiation induction method.
VI. Appendix A: Reference Pictures

![Mesoderm Booster -](image1) ![Mesoderm Booster +](image2)

Figure 1. Representative immunofluorescence images of hPSC cultures maintained under skeletal muscle differentiation conditions with (right) or without (left) Mesoderm Booster. These cultures were fixed with 4% paraformaldehyde 7 days after the initiation of differentiation. Blue fluorescence shows nuclei stained with Hoechst 33258 and red fluorescence indicates the presence of myosin heavy chain. Bars, 50 μm. This experiment was performed using Quick-Muscle™ Skeletal - SeV Complete Kit (catalogue number: EXGS-QMSSV; [https://elixirgenscientific.com/store/quick-muscle-skeletal/](https://elixirgenscientific.com/store/quick-muscle-skeletal/)) available from Elixirgen Scientific.

VII. Appendix B: Troubleshooting

<table>
<thead>
<tr>
<th>Issue</th>
<th>Possible Reason</th>
<th>Solution</th>
</tr>
</thead>
</table>
| My culture does not survive after addition of Mesoderm Booster.     | Cell density was too low to treat with Mesoderm Booster. | Plate 1.0-1.2 × 10^5 cells per well one day before treatment with Mesoderm Booster. If a user find that he or she needs a higher number of cells for plating to obtain a culture with 40-60% confluency one day after plating, it is highly likely that those cells are unhealthy or damaged during harvesting.  
| hPSCs responded poorly to Mesoderm Booster.                         |                                        | Use different hPSC lines.                                                |
| My culture has colonies of incompletely differentiated cells at the end point. | Cell density was too high to treat with Mesoderm Booster. | Plate 0.6-0.8 × 10^5 cells per well one day before treatment with Mesoderm Booster.  
| Cells were plated unevenly.                                         |                                        | Gently rock the plate several times after plating cells.                 |
| Mesoderm Booster was left at room temperature for too long.         |                                        | Use Mesoderm Booster immediately after thawing or store it at 4°C no longer than 3 hours. |
| My culture has differentiated cells but only on one side of the well. | Mesoderm Booster was not uniformly mixed with the medium. | Swirl the plate for 15 seconds after addition of Mesoderm Booster before incubation. |
VIII. Appendix C: Literature References


2. This kit is based on the technology published in (1) and licensed from Keio University.
Elixirgen Scientific provides pluripotent stem cell differentiation services with the world’s fastest turnaround time. Customers can simply ship live iPS/ES cells in a T-25 flask and will receive live or frozen cells in a week (express service) or two weeks (regular service). Contact services@elixirgenscientific.com for more details to customize for your project.

Currently Elixirgen Scientific offers all tissue types from kits for cell differentiation services. More tissue types are coming soon!