User Guide

ELIXIRGEN SCIENTIFIC

Quick-Muscle™ Skeletal - SeV Kit (Small)

Catalog Number: SM-SeV-S

Introduction

The Quick-Muscle™ Skeletal - SeV Kit facilitates rapid and efficient differentiation of human iPS or ES cells into skeletal muscle cells in just 7 days. Our proprietary transcription factor-based stem cell differentiation method uses temperature sensitive Sendai virus to produce a highly pure population of skeletal muscle cells without a genetic footprint. Quick-Muscle™ Skeletal differentiated cell cultures display typical skeletal muscle morphology and markers, such as myosin heavy chain (MHC).

Scale: The Quick-Muscle™ Skeletal - SeV Kit (Small) contains a set of reagents for use with a total of 4 wells of a 24-well plate.

Related Products: Quick-Muscle™ Skeletal - mRNA Kit, Catalog Number: SM-mRNA
Quick-Muscle™ Skeletal - Maintenance Medium, Catalog Number: SM-MM

Kit Contents

Upon receipt, store the reagents at the temperatures indicated in the table below. All reagents are shipped on dry ice.

Reagents	Volume	Storage
QMS-SeV*	110 µl	-80°C
Mesendoderm RNA	4.5 µl	-20°C or -80°C
Component P	14 µl	-20°C or -80°C
Coating Material A	15.7 µl	-20°C or -80°C

This kit contains iMatrix-511 silk (Nippi, Inc.).

***IMPORTANT!** This kit contains Sendai virus (SeV) particles that are active at 33°C and become inactive at 37°C. SeV is non-pathogenic in humans, and humans are not natural hosts of SeV; however, Biosafety Level 2 (BSL-2) containment is required for its use. Please use a biological safety cabinet, laminar flow hood, and proper personal protective equipment in order to prevent mucosal exposure. More information on BSL-2 guidelines can be found at www.cdc.gov/labs/BMBL.html.

Conditions of Use

This product is for research use only. It is not approved for use in humans or for therapeutic or diagnostic use.

Technical Support

For technical support, please contact us at cs@elixirgensci.com or call +1 (443) 869-5420 (M-F 9 am-5 pm EST).

Last revised: January 22, 2021

Required Consumables

Item	Vendor	Catalog Number
24-well tissue-culture-treated polystyrene plate (e.g., Corning Costar Flat Bottom Cell Culture Plates)	Fisher Scientific	07-200-740
Minimum Essential Medium (MEM) α, no nucleosides	ThermoFisher	12561056
KnockOut Serum Replacement	ThermoFisher	10828010
Sodium Pyruvate (100 mM)	ThermoFisher	11360070
MEM Non-Essential Amino Acids Solution (100X)	ThermoFisher	11140050
β-mercaptoethanol	ThermoFisher	21985023
Glutamax (100x)	ThermoFisher	35050061
Penicillin-Streptomycin	ThermoFisher	15140122
TrypLE Select Enzyme (1X)*	ThermoFisher	12563011
0.02% EDTA in DPBS	Sigma-Aldrich	E8008-100ML
Opti-MEM, reduced serum	ThermoFisher	31985062
Lipofectamine MessengerMax	ThermoFisher	LMRNA015
Phosphate-buffered saline (without Ca^{++} Mg^{++})	ThermoFisher	20012050
ROCK inhibitor Y27632	Selleckchem	S1049
Dimethyl sulfoxide (DMSO)	Sigma-Aldrich	D8418

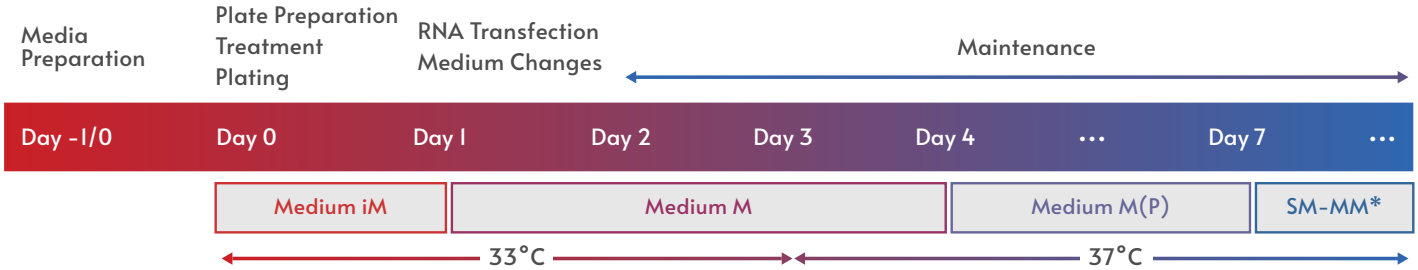
* Can be substituted with our Cell Dissociation Reagent (Solution D1), Catalog Number: CDR.

Source hPSC Culture Conditions

The Quick-Muscle™ Skeletal - SeV Kit (Small) gives the best differentiation results when source hPSCs have been maintained in StemFit® Basic04, StemFlex™ Medium, or other similar culture media which enable the maintenance of cultures by single-cell passaging. This protocol also assumes that the source hPSCs are cultured in a 35-mm culture dish or one well of a 6-well plate. If iMatrix-511 silk is routinely used as a coating substrate, prepare one culture dish or well precoated with 0.25 µg/cm² iMatrix-511 silk diluted in 2 ml chilled PBS for this kit.

- The protocols and reagents for StemFit® Basic04 and iMatrix-511 silk culture conditions are available at Elixirgen Scientific (Catalog Numbers: ASB04, NI511S).
- Differentiation should not be performed until the cells are at least 14 days post-thaw.

Workflow



*From Day 7, users may maintain differentiated cells in the maintenance medium best suited for their needs, though we recommend Quick-Muscle™ Skeletal - Maintenance Medium, Catalog Number: SM-MM.

Media Preparation

10 mM β-mercaptoethanol

1. Mix 80 µl 55 mM β-mercaptoethanol with 360 µl PBS.
2. Filter sterilize and store at 4°C.

10 mM ROCK inhibitor Y27632 (iROCK)

1. Dissolve 10 mg ROCK inhibitor Y27632 in 3.12 ml DMSO.
2. Make aliquots of a convenient volume (e.g., 100 µl).
3. This solution is hereafter referred to as iROCK and can be stored at -20°C.

0.5X TrypLE Select with EDTA (Solution D1)*

1. Mix 1 ml TrypLE Select Enzyme (1X) with 1 ml 0.02% EDTA in DPBS.
2. This mixture (hereafter referred to as Solution D1) can be stored at 4°C for 2 weeks.

*Can be substituted with our Cell Dissociation Reagent (Solution D1), Catalog Number: CDR.

Medium M

1. Prepare Medium M using the reagents listed in the table below.
    - Take out all required reagents and incubate at RT for 1 hour.
2. Store Medium M for up to 2 weeks at 4°C.

Medium M Reagents	Volume
Minimum Essential Medium (MEM) α, no nucleosides	13.5 ml
KnockOut Serum Replacement	750 µl
100 mM Sodium Pyruvate	150 µl
Non-essential amino acids (100x)	150 µl
Glutamax (100x)	150 µl
Penicillin-Streptomycin (10000 units/ml; 100x)	150 µl
10 mM β-mercaptoethanol	150 µl

Medium M(P)

1. Prepare Medium M(P) using the reagents listed in the table below.
    - Take out all required reagents and incubate at RT for 20-30 minutes.
2. Store Medium M(P) for up to 2 weeks at 4°C.

Medium M(P) Reagents	Volume
Medium M	4.2 ml
Component P	2.1 µl

Day 0

~4 hr

Plate Preparation

1. Prepare diluted Coating Material A by mixing together the following components in a 15 ml conical tube.
   - Thaw Coating Material A on ice for 20-30 minutes (or at 4°C overnight one day before Day 0).
   - Make sure chilled PBS is used for this mixture.

Diluted Coating Material A Reagents	Volume
Coating Material A	6.6 µl
Chilled PBS	2 ml

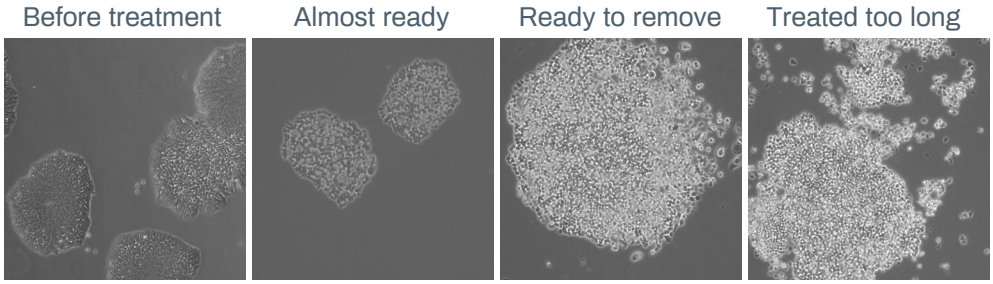
2. Add 400 µl diluted Coating Material A to each new well of 4 wells.
3. Incubate the plate at 37°C, 5% CO_2 for 2 hours (or 4°C overnight one day before Day 0).
4. Aspirate the supernatant from each well and add 500 µl PBS.
5. Incubate the plate at 37°C, 5% CO_2 until hPSCs are ready for plating.

Treatment

1. Prepare Medium iM by mixing together the following components in a 15 ml conical tube.
   - Warm Medium M, iROCK, and Solution D1 at room temperature for at least 1 hour.
   - The rest of Medium M should be stored at 4°C for later use.

Medium iM Reagents	Volume
Medium M	2.5 ml
iROCK	2.5 µl

2. Thaw QMS-SeV on ice.
3. Tap the Solution D1 tube 5 times with a finger and centrifuge at maximum speed for 1 minute.
4. Aspirate old medium from hPSC culture and add 2 ml PBS.
5. Rock the plate 3 times, aspirate PBS from the culture, and add 300 µl of the cell dissociation reagent Solution D1. Keep the rest of Solution D1 at 4°C for use on Day 3.
6. Incubate the culture at 37°C, 5% CO_2 for 5 minutes. If all the cells are not rounded under a microscope, incubate at 37°C, 5% CO_2 for up to 5 more minutes in 1-2 minute increments (see images below).



7. Carefully pipet out Solution D1 from the culture using a P1000 pipettor and add 1 ml Medium iM to the well.
8. Disperse the medium over the bottom surface of the well by pipetting 8-15 times to detach cells.
9. Using the same pipette tip, collect the cell suspension in a 1.5 ml tube.

Plating

1. Following this user guide, users will plate the hPSCs into 4 wells with 250 µl Medium iM per well. Please refer to the table on the next page for our plating recommendations for each of the 4 wells. If the volume of the cell suspension needed to get the desired cell density is greater than 250 µl, centrifuge the required volume of cell suspension at 200xg for 4 minutes, remove the supernatant, and resuspend the pellet into 250 µl Medium iM.
2. Working on one well at a time, pipet out PBS from one of the four coated wells using a P1000 pipettor.
3. Immediately add 250 µl cell suspension into the well using a P1000 pipettor.
4. Repeat Step 2 and 3 for the rest of the cell suspensions.

IMPORTANT! Before adding QMS-SeV, ensure that it is fully thawed. Do not centrifuge, vortex, or mix SeV with a pipettor; SeV is highly sensitive to physical stress.

5. Add the indicated volume of QMS-SeV into each well using a P200 pipettor.
    - By pushing the plunger, a small drop should be formed. As the drop is formed it can touch the medium surface. Repeat this process by making several drops to touch, e.g., 12, 3, 6 and 9 o'clock of the medium surface or until the tip becomes empty.

Cell Density	QMS-SeV
0.8 x 10^5 cells	20 µl
1.0 x 10^5 cells	25 µl
1.2 x 10^5 cells	30 µl
1.4 x 10^5 cells	35 µl

6. Incubate the cultures at 33°C, 5% CO_2 overnight.

Day 1

4-8 hr

IMPORTANT! The duration of incubation after the medium change is critical for the second treatment. If the confluency of any of the cultures is between 50-70%, the first treatment can be started anytime (even immediately after medium change) up to 1 hour after the medium change. However, if any of the cultures have less than 50% confluency, the cultures should be incubated up to 4 hours. If the cultures have greater than 70% confluency, the first treatment should be done within 1 hour after the medium change, but the differentiation efficiency is expected to be low.

Medium Change

1. Warm Medium M at room temperature for 20-30 minutes.
2. Pipet out the old medium from each well and add 250 µl Medium M to each well using a P1000 pipettor.
3. Incubate the cultures at 33°C, 5% CO_2 for 1-4 hours (see the note above).

Mesendoderm RNA Transfection

1. 45 minutes before the above incubation is completed, begin warming/thawing the following reagents.
    - Thaw Mesendoderm RNA on ice for 30 minutes.
    - Warm Opti-MEM at RT for 20-30 minutes.
    - Take Lipofectamine MessengerMAX (LMM) out from the refrigerator and leave on ice.
2. Take 110 µl Opti-MEM and add it to two 1.5 ml tubes using a P200 pipettor.
3. Add 4.5 µl LMM to one of the 1.5 ml tubes with 110 µl Opti-MEM using a P10 pipettor and mix it by vortexing at medium speed for 5 seconds.
4. Label it as Mix 1 and leave it at RT for 10 minutes.
5. IMPORTANT! Immediately before 10 minutes pass (i.e., around 8 minutes), add 4.5 µl Mesendoderm RNA to the other 1.5 ml tube with 110 µl Opti-MEM using a P10 pipettor and label it as Mix 2. Mix by tapping the tube 5 times.
    - Keep the rest of LMM in the refrigerator. Do not leave it at RT.

Mix 1 Reagents	Volume
Opti-MEM	110 µl
LMM	4.5 µl

Mix 2 Reagents	Volume
Opti-MEM	110 µl
Mesendoderm RNA	4.5 µl

6. 10 minutes after making Mix 1, add Mix 2 into Mix 1 using a P200 pipettor, and pipet it up and down 8-10 times. The resulting mixture is called Mesendoderm Booster.
7. Leave Mesendoderm Booster at RT for 5 minutes.
8. Working one well at a time, add 52 µl Mesendoderm Booster to each well by making a small drop at the tip of the pipettor with one hand while gently shaking the plate with the other hand. As the drop is formed it can touch the medium surface. Repeat this process by making several drops to touch, e.g., 12, 3, 6 and 9 o'clock of the medium surface or until the tip becomes empty.
9. Swirl the plate for 15 seconds to evenly distribute Mesendoderm Booster in the wells.
10. Incubate the cultures at 33°C, 5% CO_2 for 3 hours.

Medium Change

1. Warm Medium M at room temperature for 20-30 minutes.
2. Pipet out the old medium from all wells using a P1000 pipettor.
3. Add 500 µl Medium M to each well using a P1000 pipettor.
4. Incubate the cultures at 33°C, 5% CO_2 overnight.

Day 2

<1 hr

Maintenance

1. Warm Medium M at room temperature for 20-30 minutes.
2. Pipet out the old medium from each well using a P1000 pipettor, leaving a small volume behind to avoid the cells drying out.
3. Add 500 µl Medium M to each well using a P1000 pipettor.
4. Incubate the cultures at 33°C, 5% CO_2 overnight.

Day 3

<1 hr

Maintenance and Temperature Shift

1. Warm Medium M at room temperature for 20-30 minutes.
2. Pipet out the old medium from each well using a P1000 pipettor, leaving a small volume behind to avoid the cells drying out.
3. Add 500 µl Medium M to each well using a P1000 pipettor.
4. Incubate the cultures at 37°C, 5% CO_2 overnight.

Day 4

<1 hr

Maintenance

1. Warm Medium M(P) at room temperature for 20-30 minutes.
2. Pipet out the old medium from each well using a P1000 pipettor, leaving a small volume behind to avoid the cells drying out.
3. Add 1 ml Medium M(P) using a 5 ml pipettor.
4. Incubate the cultures at 37°C, 5% CO_2 for 3 days.

Day 7

Assay or Continuous Maturation

Elongated, spindle-shaped muscle cells can be observed on Day 4. For more mature skeletal muscle, we recommend culturing cells until Day 7. From Day 7, users may maintain differentiated skeletal muscle in the maintenance medium best suited for their needs, though we recommend Quick-Muscle™ Skeletal - Maintenance Medium, Catalog Number: SM-MM. Differentiation into skeletal muscle can be confirmed with an anti-MHC (myosin heavy chain) antibody.

Appendix

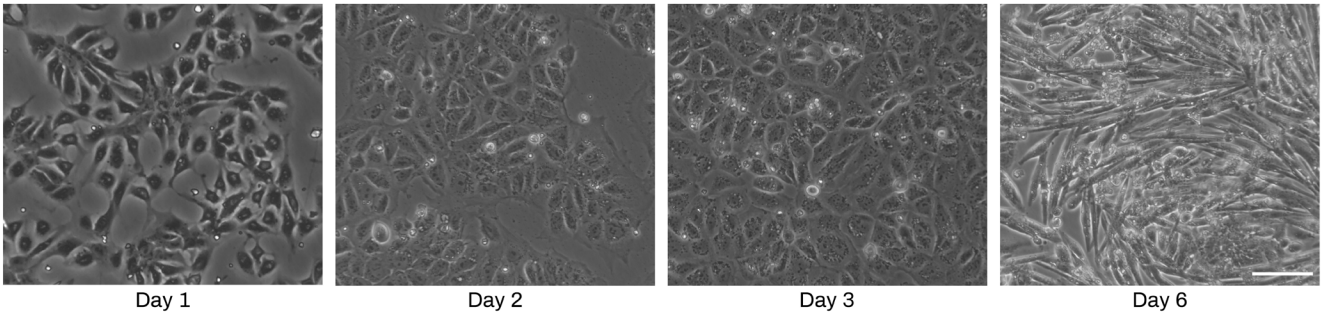


Figure 1. Representative images of Quick-Muscle™ Skeletal - SeV Kit cell cultures on days 1-6 post-differentiation (scale bar = 100 µm).

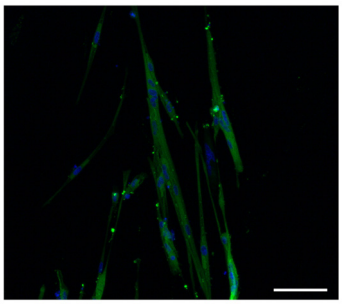


Figure 2. Immunofluorescent staining of Quick-Muscle™ Skeletal - SeV cell cultures on day 15 shows typical skeletal muscle morphology and expression of myosin heavy chain (MHC). Nuclei were counterstained with Hoechst 33324.