User Guide

Quick-Muscle™ Skeletal - mRNA Kit (Small)

Catalog Number: SM-mRNA-S

Introduction

The Quick-Muscle™ Skeletal - mRNA Kit (Small) 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 synthetic mRNAs to produce myosin heavy chain (MHC)-positive muscle cells without leaving a genetic footprint.

Scale: The Quick-Muscle™ Skeletal - mRNA 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 - SeV Kit (Small) , Catalog Number: SM-SeV
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
QM1-mRNA	9 µl (4.4 µg)	-80°C
QM2-mRNA	9 µl (4.4 µg) x 3	-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.).

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
Lipofectamine MessengerMAX	ThermoFisher	LMRNA001
Opti-MEM I Reduced Serum Medium	ThermoFisher	31985062
Minimum Essential Media (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
Glutamax (100x)	ThermoFisher	35050061
Penicillin-Streptomycin	ThermoFisher	15140122
β-mercaptoethanol (β-ME)	ThermoFisher	21985023
StemFit Basic04 Complete Type, or StemFit Basic04, or StemFit Basic02, or StemFlex Medium	Elixirgen Scientific Elixirgen Scientific Elixirgen Scientific ThermoFisher	ASB04-C, or ASB04-F, or ASB02, or A3349401
TrypLE Select Enzyme (1X)*	ThermoFisher	12563011
0.02% EDTA in DPBS	Sigma-Aldrich	E8008-100ML
Phosphate-buffered saline (without Ca^{++} Mg^{++})	ThermoFisher	20012050
ROCK inhibitor Y27632	Selleckchem	S1049
Dimethyl sulfoxide (DMSO)	Sigma-Aldrich	D8418

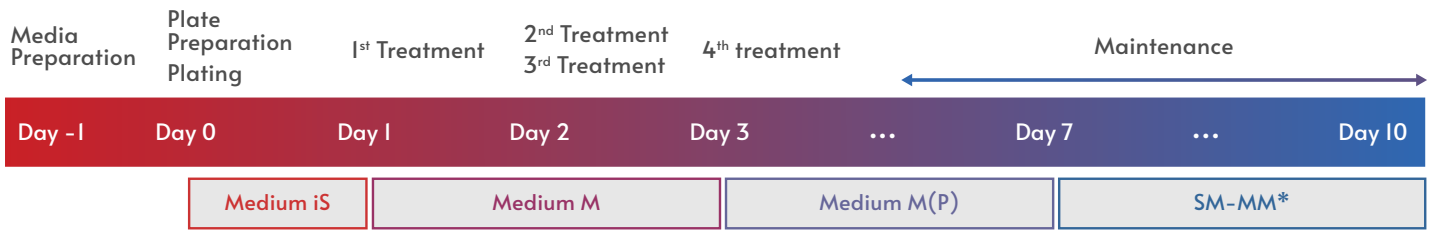
* Can be substituted with our Cell Dissociation Reagent (Solution D1), Catalog Number: CDR.

Source hPSC Culture Conditions

The Quick-Muscle™ Skeletal - mRNA Kit (Small) gives 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

IMPORTANT! For the best possible delivery of QM1-mRNA and QM2-mRNA into cells, we recommend Lipofectamine MessengerMax. If users prefer another transfection reagent, please make sure that the reagent provides a transfection efficiency of ≥80% prior to using this kit. QM1-mRNA or QM2-mRNA mixed with Lipofectamine MessengerMax must be immediately applied to cultures and cannot be stored.

10 mM β-ME

1. Mix 80 µl 55 mM β-ME 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.

StemFit Basic04 Complete Type (Medium S)*

1. Thaw StemFit Basic04 Complete Type bottle overnight or multiple nights at 4°C.
2. Make aliquots of a convenient volume (e.g., 40 ml).
3. This solution is hereafter referred to as Medium S and can be stored at -80°C.

*Medium S can be substituted with StemFit Basic02/04 (with bFGF added) or StemFlex.

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.
    - Thaw all reagents at room temperature for 20-30 minutes.
2. Store Medium M for up to 2 weeks at 4°C. The leftover reagents can be discarded or saved for other uses.

Medium M Reagents	Volume
Minimum Essential Media (MEM) α, no nucleosides	18 ml
KnockOut Serum Replacement	1 ml
Sodium Pyruvate (100 mM)	200 µl
MEM Non-Essential Amino Acids Solution (100X)	200 µl
200 mM Glutamax (100x)	200 µl
Penicillin-Streptomycin (10000 units/ml; 100x)	200 µl
10 mM β-ME	200 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 room temperature for 20-30 minutes.
2. Store Medium M(P) for up to 2 weeks at 4°C.

Medium M(P) Reagents	Volume
Medium M	9 ml
Component P	4.5 µl

Day 0

~3 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	11.9 µl
Chilled PBS	3.6 ml

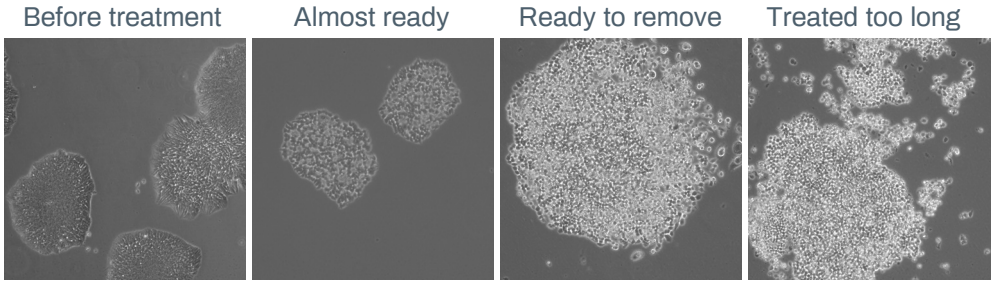
2. Add 400 µl diluted Coating Material A to each new well of 8 wells.
3. Incubate the plate at 37°C, 5% CO_2 for 2 hours (or 4°C overnight one day before Day 0).
4. Aspirate the supernatant from each well and add 500 µl PBS.
5. Incubate the plate at 37°C, 5% CO_2 until the hPSCs are ready for plating.

Plating

1. Prepare Medium iS by mixing together the following components in a 15 ml conical tube.
    - Warm Medium S, iROCK, and Solution D1 at room temperature for at least 1 hour protected from light.

Medium iS Reagents	Volume
Medium S	5.5 ml
iROCK	5.5 µl

2. Tap the Solution D1 tube 5 times with a finger and centrifuge at maximum speed for 1 minute.
3. Aspirate old medium from hPSC culture and add 2 ml PBS.
4. Rock the plate 3 times, aspirate PBS from the culture, and add 300 µl of the cell dissociation reagent Solution D1.
5. Incubate the plate 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).



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

IMPORTANT! In this protocol, users will plate the hPSCs into 8 wells with 500 µl Medium iS per well. However, if users know the best plating density already, prepare a cell suspension enough to plate 5 wells (4 wells plus 1 extra to account for the pipetting/plating error). We recommend aiming for 50-70% initial cell confluency on Day 1, with 20-50 cells per colony. Our data indicate that cell counts ranging from 1.0 - 2.4 x 10^5 viable cells per well are suitable. Cell count may vary based on cell health and the method used for cell counting.

If the confluency on Day 1 is
- above the target range, the differentiation efficiency will decrease.
- below the target range, more cell death will be observed.

Note continued on next page

For first time users, we recommend plating the following numbers of cells into each of 8 wells: 1.0, 1.2, 1.4, 1.6, 1.8, 2.0, 2.2 and 2.4 x 10^5 cells. On Day 1, 4 wells showing 50-70% confluency should be selected for transfection.

To those who know the cell number that shows 50-70% confluency on Day 1 after plating, the range of cell numbers can be narrower and more specific. For example, if you already know plating 1.2 - 1.4 x 10^5 live cells will result in 50-70% confluency, our recommendation for preparing 8 wells is as follows: 1.0, 1.1, 1.2, 1.3, 1.4, 1.5, 1.6 and 1.7 x 10^5 live cells.

9. Take out the volume of the cell suspension needed for each cell density, according to the note above, and place each in a new tube labeled with the corresponding density.
10. Bring the volume of the cell suspension in each tube up to 500 µl with Medium iS.
    - If the volume in the tube exceeds 500 µl, centrifuge the required volume of cell suspension at 200xg for 4 minutes, remove the supernatant, and resuspend the pellet in 500 µl Medium iS.
11. Aspirate PBS from each coated well and add 500 µl cell suspension to each well using a P1000 pipettor.
12. Place the plate in the incubator and rock it front to back and side to side for 15 seconds to make sure that the cells are evenly distributed.
13. Incubate the cultures at 37°C, 5% CO_2 overnight.

Day 1

~5-8 hr

IMPORTANT! In this protocol, users will identify the best 4 wells with confluency of 50-70% after replacing Medium iS with Medium M in the morning. After the medium change, cells will exhibit a more flattened shape, thereby increasing the confluency. The duration of incubation after the medium change is critical for the first treatment. If users can select 4 cultures having a confluency falling 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 all of the cultures have less than 50% confluency, the cultures can be incubated up to 4 hours until they start to exhibit 50-70% confluency. If all of the cultures have greater than 70% confluency, the first treatment should be done immediately after the medium change, although the differentiation efficiency is expected to be low.

First Treatment

1. Thaw 1 vial of QM1-mRNA on ice for 30 minutes and warm Opti-MEM and Medium M at room temperature for 20-30 minutes.
2. According to the note above, select 4 cultures with 50-70% confluency. Aspirate the old medium from each well and add 500 µl Medium M. Repeat this process for each of the selected 4 wells. Do not let cells dry out during the medium change.
3. Incubate the cultures at 37°C, 5% CO_2 as needed.
4. Prepare QM1 by the following steps:
    - Prepare two 1.5 ml tubes with 220 µl Opti-MEM each.
    - Add 9 µl LMM to one of the 1.5 ml tubes and mix by brief vortexing. Leave it at room temperature for 10 minutes (Mix 1). Keep the rest of LMM at 4°C for later treatments.
    - **IMPORTANT!** Immediately before 10 minutes pass (i.e., around 8 minutes), add 9 µl QM1-mRNA to the other 1.5 ml tube with Opti-MEM (Mix 2). Mix by tapping 5 times. Do not vortex.
    - 10 minutes after mixing LMM with Opti-MEM, add Mix 2 into Mix 1, and pipet up and down 8-10 times. This mixture is called QM1. Leave QM1 at room temperature for 5 minutes and no longer.

Mix 1 Reagents	Volume
Opti-MEM	220 µl
LMM	9 µl

Mix 2 Reagents	Volume
Opti-MEM	220 µl
QM1-mRNA	9 µl

5. Add 104 µl QM1 to each culture by adding QM1 dropwise with one hand while gently shaking the plate with the other hand.
6. Rock the plate front to back and side to side for 15 seconds to make sure that QM1 is evenly distributed in the cultures.
7. Incubate the cultures at 37°C, 5% CO_2 for 2.5-3 hours.
8. Pipet out the medium with QM1 from each well using a P1000 pipettor and add 500 µl Medium M.
9. Incubate the cultures at 37°C, 5% CO_2 overnight.

Day 2

~8 hr

Second Treatment

1. Observe the QM1-treated cultures to ensure they are at 60-80% confluency before proceeding. If the cultures have a lower confluency than 60%, cell death is expected at some degree after the following treatments. If the cultures have a greater confluency than 80%, then the differentiation efficiency is expected to be lower.
2. Thaw 1 vial of QM2-mRNA on ice for 30 minutes and warm Opti-MEM and Medium M at room temperature for 20-30 minutes.
3. Pipet out the medium from each of the 4 wells using a P1000 pipettor and add 500 µl Medium M.
4. Incubate the cultures at 37°C, 5% CO_2 for at least 10 minutes.
5. Prepare QM2 by the following steps:
    - Prepare two 1.5 ml tubes with 220 µl Opti-MEM each.
    - Add 9 µl LMM to one of the 1.5 ml tubes and mix by brief vortexing. Leave it at room temperature for 10 minutes (Mix 1). Keep the rest of LMM at 4°C for later treatments.
    - **IMPORTANT!** Immediately before 10 minutes pass (i.e., around 8 minutes), add 9 µl QM2-mRNA to the other 1.5 ml tube with Opti-MEM (Mix 2). Mix by tapping 5 times. Do not vortex.
    - 10 minutes after mixing LMM with Opti-MEM, add Mix 2 into Mix 1, and pipet up and down 8-10 times. This mixture is called QM2. Leave QM2 at room temperature for 5 minutes and no longer.

Mix 1 Reagents	Volume
Opti-MEM	220 µl
LMM	9 µl

Mix 2 Reagents	Volume
Opti-MEM	220 µl
QM2-mRNA	9 µl

6. Add 104 µl QM2 to each culture by adding QM2 dropwise with one hand while gently shaking the plate with the other hand.
7. Rock the plate front to back and side to side for 15 seconds to make sure that QM2 is evenly distributed in the cultures.
8. Incubate the cultures at 37°C, 5% CO_2 for 2.5-3 hours.

Third treatment

1. Pipet out the medium from each of 4 wells using a P1000 pipettor and add 500 µl Medium M to the wells.
2. Incubate the cultures at 37°C, 5% CO_2 for 2 hours.
    - Put 1 vial of QM2-mRNA on ice when the incubation above reaches 1.5 hours and leave it for 30 minutes.
3. Repeat Steps 5-8 of the “Second Treatment” section.
4. Pipet out the medium from each well using a P1000 pipettor and add 500 µl Medium M to it.
5. Incubate the cultures at 37°C, 5% CO_2 overnight.

Day 3

~3 hr

Fourth treatment

1. Observe the QM2-treated cultures to ensure they are reaching almost 100% confluency before proceeding. If the cultures have a significantly lower confluency, e.g., less than 50%, cell death has been induced. If the cultures have grown over confluent, the differentiation efficiency is expected to be lower.
2. Thaw 1 vial of QM2-mRNA on ice for 30 minutes and warm Opti-MEM and Medium M at room temperature for 20-30 minutes.
3. Pipet out the medium from each of the 4 wells using a P1000 pipettor and add 500 µl Medium M.
4. Incubate the cultures at 37°C, 5% CO_2 for at least 10 minutes.
5. Repeat Steps 5-8 of the “Second Treatment” section.
6. Warm Medium M(P) at room temperature for 20-30 minutes.
7. Pipet out the medium with QM2 from each well using a P1000 pipettor and add 500 µl Medium M(P) to it.
8. Incubate the cultures at 37°C, 5% CO_2 overnight.

Day 4

<1 hr

Maintenance

1. Observe the cultures under a microscope and confirm the presence of elongated, spindle-shaped cells in the culture. A certain degree of cell death is expected.
2. Warm Medium M(P) at room temperature for 20-30 minutes.
3. Pipet out the old medium from each well using a P1000 pipettor and add 1 ml Medium M(P).
4. Incubate the cultures at 37°C, 5% CO_2 for 3 days.

Day 7

Assay or Continuous Maturation

Elongated, spindle-shaped 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 cultures 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 after using the Quick-Muscle™ Skeletal - mRNA Kit can be confirmed with an anti-MHC (myosin heavy chain, a marker for skeletal muscle) antibody.

Appendix

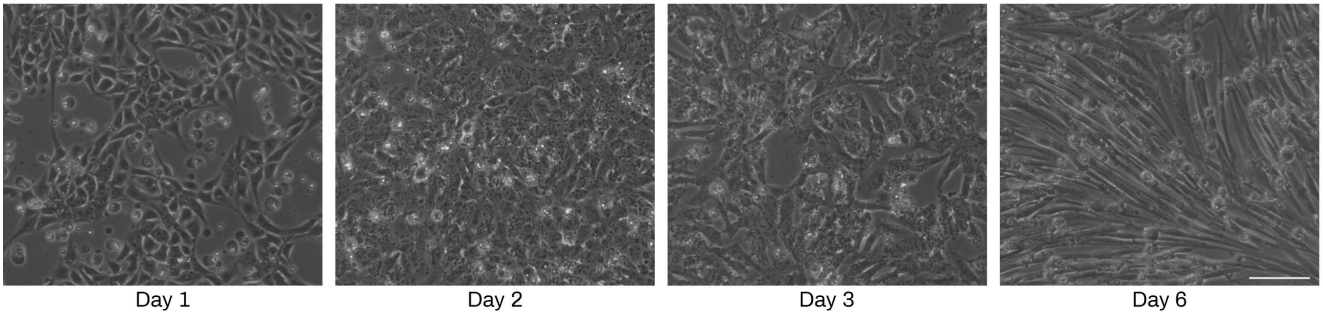


Figure 1. Representative images of Quick-Muscle™ Skeletal - mRNA Kit cell cultures on days 1-6 post-differentiation (scale bar = 100 µm).

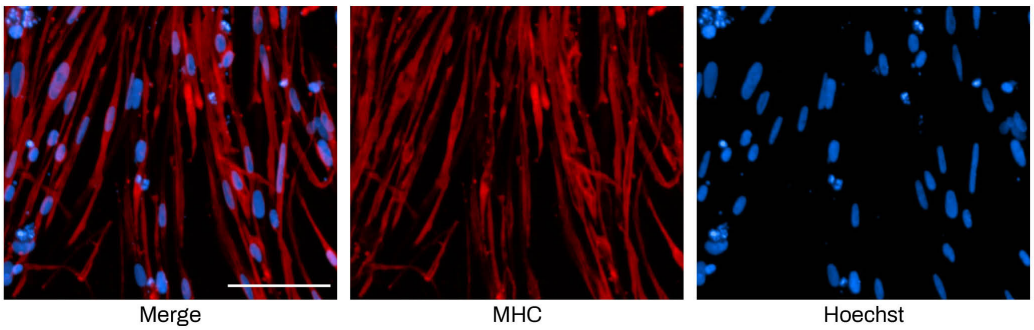


Figure 2. Immunofluorescent staining of Quick-Muscle™ Skeletal - mRNA cell cultures shows typical skeletal muscle morphology and expression of myosin heavy chain (MHC) on day 6 post-differentiation. Staining conditions: Anti-MHC primary antibody (R&D Systems, Catalog Number: MAB4470, 1:250 dilution) in combination with a secondary antibody Goat anti-Mouse IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor 594 (Invitrogen,Catalog Number: A11032 , 1:500 dilution). Nuclei were counterstained with Hoechst 33324.