# **User Guide**



Ricoh Biosciences, Inc.

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# Quick-Muscle™ Skeletal - Maintenance Medium

Catalog Number: SM-MM

# Introduction

Quick-Muscle™ Skeletal - Maintenance Medium may be used for the long-term maintenance of human pluripotent stem cell-derived skeletal muscle cells following differentiation as outlined in the Quick-Muscle™ Skeletal - SeV Kit user guides. Quick-Muscle™ Skeletal differentiated cell cultures display typical skeletal muscle morphology and markers, such as myosin heavy chain (MHC). When handled and maintained according to the instructions in this user guide, skeletal muscle cells are viable long-term.

Scale: The Quick-Muscle™ Skeletal - Maintenance Medium provides sufficient medium for 4 wells of a

24-well plate, 1 well of a 6-well plate, or 16 wells of a 96-well plate for up to 2 weeks.

Related Products: Quick-Muscle™ Skeletal - SeV Kit, Catalog Number: SM-SeV

### **Contents**

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

Contents	Volume	Storage	Thaw
Component M1	750 μl	-20°C or -80°C	On ice or 4°C
Component P	50 µl	-20°C or -80°C	Room temperature

## **Condition 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 refer to the FAQ on our website.

You may also contact us at cs@biosciences.ricoh.com or call +1 (443) 869-5420 (M-F 9am-5pm EST).

# **Required Consumables**

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Item	Vendor	Catalog Number
DMEM/F12	ThermoFisher	21331020
GlutaMAX	ThermoFisher	35050061
Penicillin-Streptomycin	ThermoFisher	15140122

# **Preparation**

#### Medium M1

- 1. Prepare Medium M1 using the reagents listed in the table below.
  - Thaw Component M1 for 20-30 minutes at the temperature indicated in the "Contents" table on page 1.
  - Warm all other reagents at room temperature for 20-30 minutes.
  - o Tap the Component M1 tube 3 times and then briefly spin it down before use.
  - o Keep Medium M1, and any subsequent media made with it, protected from light.
  - Store Medium M1 for up to 2 weeks at 4°C.
  - Leftover Component M1 can be discarded or saved at 4°C for up to two weeks.

Reagents	Volume
DMEM/F12	14 ml
GlutaMAX	147.6 µl
Penicillin-Streptomycin (10000 units/ml; 100x)	147.6 µl
Component M1	443 µl

## **First Week**

- 1. Prepare Medium M1(P) using the reagents listed in the table below.
  - o Thaw Component P for 20-30 minutes at the temperature indicated in the "Contents" table on page 1.
  - Warm all other reagents at room temperature for 20-30 minutes.
  - o Tap the Component P tube 3 times and then briefly spin it down before use.
  - Store Medium M1(P) for up to 2 weeks at 4°C.
  - Leftover Component P can be saved at 4°C.

Reagents	Volume
Medium M1	7 ml
Component P	3.5 µl

2. Pipet out most of the old medium, but not completely (i.e., just enough to cover the surface of the well), from each well and very slowly along the wall of the well, add Medium M1(P) according to the following table.

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	Red	Required volume per well		
Reagents	6-well plate	24-well plate	96-well plate	
Medium M1(P)	2 ml	800 µl	150 µl	

- 3. Incubate the cultures at 37°C, 5% CO<sub>2</sub> for 2 days.
- 4. For subsequent medium changes, pipet out half (see volumes in the table above) of the old medium from each well and replace with an equal volume of room temperature Medium M1(P).
- 5. Repeat Step 4 every 2-3 days such as on Monday, Wednesday, and Friday for 1 week.

# **Second Week**

- 1. Warm Medium M1 at room temperature for 20-30 minutes until it no longer feels cold.
- 2. Pipet out most of the old medium, but not completely (i.e., just enough to cover the surface of the well), from each well and very slowly along the wall of the well, add Medium M1 according to the following table.

	Rec	Required volume per well		
Reagents	6-well plate	24-well plate	96-well plate	
Medium M1	2 ml	800 µl	150 µl	

- 3. Incubate the cultures at  $37^{\circ}$ C, 5% CO<sub>2</sub> for 2 days.
- 4. For subsequent medium changes, pipet out half (see volumes in the table above) of the old medium from each well and replace with an equal volume of room temperature Medium M1.
- 5. Repeat Step 4 every 2-3 days such as on Monday, Wednesday, and Friday for 1 week.

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