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Quick-Tissue™ Adaptation Kit

Catalog Number: EXGS-QTA1

User Manual

For adaption of human pluripotent stem cells (hPSCs) cultured in non-StemFit® conditions into hPSCs suitable for Quick-Tissue™ differentiation kits

This kit works for both human embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs)

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I. Introduction

The Quick-Tissue™ kits give their best differentiation results when the source hPSCs are routinely maintained in StemFit® Basic02 (StemFit®) culture medium. If the user's current hPSC culture conditions are different from StemFit® conditions, we strongly recommend that users adapt their culture conditions using the Quick-Tissue™ Adaptation Kit. Once the following protocol is performed, hPSCs can be routinely maintained under StemFit® conditions or are ready to be used for a Quick-Tissue™ kit.

NOTE: EXPERIMENT SCALE

This kit contains enough reagents to adapt hPSCs on 35-mm dishes or a 6-well plate. In this protocol, instructions are described for 35-mm dishes. For other well sizes, multiply the protocol's recipe by the ratio between well sizes.

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

Reagents	Amount	Storage Conditions
Medium S	21 ml x 2	-20 °C
Solution D1	1 ml	-20 °C
Coating Material A	15.7 µl	-20 °C

- This kit contains StemFit® Basic02 (Ajinomoto Co., Inc.) and iMatrix-511 silk (Nippi, Inc.).

III. Additional Materials Required

The following materials are needed but not supplied with this kit:

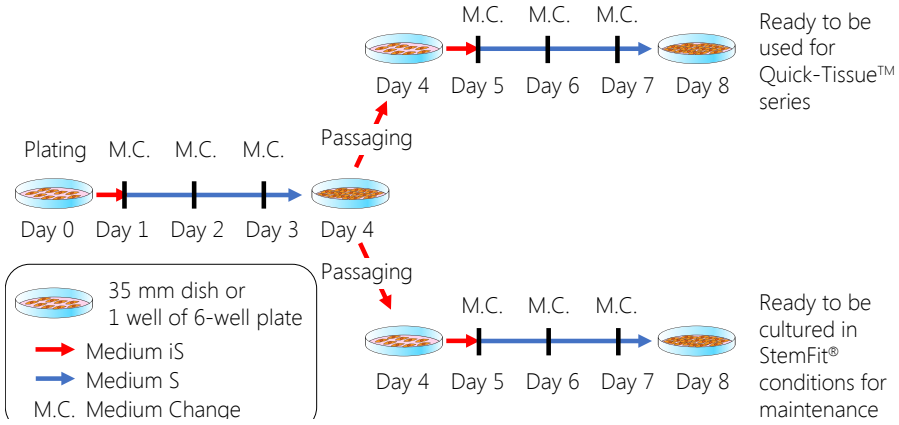
- Phosphate-buffered saline (PBS without Ca++ Mg++)
- ROCK inhibitor Y27632 (e.g., Selleckchem Catalog Number: s1049)
- Dimethyl sulfoxide (DMSO; e.g., Sigma-Aldrich, Catalog Number: D8418)
- Three 35-mm dishes or one 6-well plate

IV. Pre-Protocol Preparation

- This protocol requires one CO_2 incubators at 37°C.
- Prepare a 10 mM ROCK inhibitor Y27632 stock solution in DMSO to prepare for Medium iS on Day 0. Preparation steps are as follows:
  - Dissolve 10 mg ROCK inhibitor Y27632 in 3.1225 ml DMSO.
  - Store at -20°C.
- We do not recommend additional freeze-thaw cycles of any reagents.
- Taking 4x and/or 10x images of cultures every day (or even after every medium change) is a good way to monitor your experiment.
- There might be some precipitates in media noticed after thawing and mixing them. However, the precipitates don't affect the quality of the media. Let the precipitates settle down for 20 minutes and take out the supernatant in a fresh tube to use throughout the protocol.

- Customer service on the phone and through email (cs@elixirgenscientific.com) is available to assist users in troubleshooting and interpretation of results.

IV. Protocol



Day 0 - Plating

New Plate Preparation

1. Start thawing a bottle of Medium S at room temperature. Make sure that Medium S is at room temperature for at least 1 hour before use.
2. Thaw Coating Material A on ice for 20-30 minutes (or at 4°C overnight one day before Day 0).
3. Take 2 ml ice-cold PBS into a tube and add 4.75 µl Coating Material A to it. Mix them well. Store the rest Coating Material A at 4°C for its use on Day 4.
4. Add 2 ml diluted Coating Material A to a 35-mm dish.
5. Incubate the dish at 37°C, 5% CO_2 for 2 hours (or 4°C overnight one day before Day 0).
6. Aspirate the supernatant from the dish and add 2 ml PBS to it.
7. Incubate the dish at 37°C, 5% CO_2 until user's hPSCs are ready for plating.

Plating

1. Take 12 ml Medium S into a 15 ml conical tube and add 12 µl 10 mM ROCK inhibitor Y27632 to it. Mix them well. This medium is referred to as Medium iS. The rest of Medium S should be stored at 4°C for later use.
2. Proceed hPSC dissociation by the user's current method.
3. Carefully pipet out cell-dissociation enzyme from the culture using a P1000 pipettor and add 1 ml Medium iS to it.
4. Disperse the medium over the well bottom surface by pipetting 8-15 times to detach cells.
5. Collect the cell suspension in a tube..

NOTE: CELL DENSITY

- If a single-cell suspension is prepared, count cells and plate $1.5x10^5$ cells per dish.
- If clumps of cells are prepared, the plating density depends on the confluency of the culture harvested.
  - If the culture shows about 80% confluency, use 100 µl of the suspension prepared at step 5 for plating.
  - If the culture shows about 30% confluency, use 333 µl of the suspension prepared at step 5 for plating.

6. Count cells to determine the volume of cell suspension needed according to the note above.
7. Take out the determined volume of the cell suspension from the previous step in a tube and bring up the

volume to 3 ml with Medium iS. The rest of Medium iS should be stored at 4°C for its use on Day 4.

8. Aspirate PBS from the coated dish and add 3 ml cell suspension to it.
9. Incubate the culture at 37°C, 5% CO_2 for 1 hour.
10. Observe the dish under a microscope to make sure that the cells are evenly distributed in the well.
11. Incubate the culture at 37°C, 5% CO_2 overnight.

Day 1 - Feeding

1. Warm Medium S for 20-30 minutes to room temperature.
2. Mark colonies with the appearance of differentiated cells if found.
3. Aspirate the old medium from the dish (and colonies of differentiated cells marked at the previous step) and add 3 ml Medium S to it.
4. Incubate the culture at 37°C, 5% CO_2 overnight.

Day 2 - Feeding

1. Warm Medium S for 20-30 minutes to room temperature.
2. Remove colonies with the appearance of differentiated cells if found.
3. Aspirate the old medium from the dish and add 3 ml Medium S to it.
4. Incubate the culture at 37°C, 5% CO_2 overnight.

Day 3 - Feeding

1. Warm Medium S for 20-30 minutes to room temperature.
2. Remove colonies with the appearance of differentiated cells if found.
3. Aspirate the old medium from the dish and add 3 ml Medium S to it.
4. Incubate the culture at 37°C, 5% CO_2 overnight.
5. Start thawing the second bottle of Medium S at 4°C overnight.

Day 4 - Passaging

1. Prepare two new 35-mm dishes as described in "New Plate Preparation" on Day 0.
2. Thaw Solution D1 and warm it for 20-30 minutes to room temperature.
3. Warm Medium iS for 20-30 minutes to room temperature.
4. Aspirate the old medium from the dish and add 1.5 ml Medium iS to it.
5. Incubate the culture at 37°C, 5% CO_2 for 1 hour.

NOTE: CELL CONFLUENCY

- hPSC culture is best for passaging when the cell confluency reaches 80-90%. However, when the growing colonies start to touch each other, consider harvesting them even if the cell confluency is below 80%. Otherwise, cells begin to differentiate.

6. Aspirate the old medium from the culture and add 2 ml PBS to it.
7. Rock the plate 3 times, aspirate PBS from the culture, and add 300 µl Solution D1 to it.
8. Incubate the culture at 37°C, 5% CO_2 for 5-7 minutes.
9. Carefully pipet out Solution D1 from the culture using a P1000 pipettor and add 1 ml Medium iS to it.
10. Disperse the medium over the well bottom surface by pipetting 8-15 times to detach cells.

11. Collect the cell suspension in a tube.
12. Bring up the cell suspension volume to 6 ml with Medium iS.
13. Aspirate PBS from each coated dish and add 3 ml cell suspension to it.
14. Incubate the cultures at 37°C, 5% CO_2 for 1 hour.
15. Observe each well under a microscope to make sure that the cells are evenly distributed in the well.
16. Incubate the cultures at 37°C, 5% CO_2 overnight.

Day 5-7 - Feeding

1. Warm Medium S for 20-30 minutes to room temperature.
2. Remove colonies with the appearance of differentiated cells if found.
3. Aspirate the old medium from each dish and add 3 ml Medium S to it.
4. Incubate the culture at 37°C, 5% CO_2 overnight.
5. Repeat steps 1-4 every day until Day 8.

Day 8 - The culture is ready for use with a Quick-Tissue™ kit

1. One 35-mm dish culture can be used for any Quick-Tissue™ kit, and the other 35-mm dish culture can be used for further maintenance of hPSCs in the StemFit® Basic02 conditions.

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Category	Product	Request Quote (Catalog number)		
		SeV Complete Kit	mRNA Complete Kit	Maintenance Medium
Quick-Tissue™ Series Differentiation Kit (mainly for 4 wells of 24-well plate)	Quick-Endothelium™ Vascular with Optional Drug Selection		$399 (EXGS-QEV)	$229 (EXGS-QEVM)
	Quick-Trilineage™ Differentiation Kit*	$549 (EXGS-Q3D)		
	Quick-Neuron™ Mixed	$349 (EXGS-QNMSV)		$129 (EXGS-QNMM)
	Quick-Neuron™ Cholinergic	$349 (EXGS-QNCSV)	$299 (EXGS-QNC)	$129 (EXGS-QNCM)
		$999 (EXGS-QNCSV96)**		
	Quick-Neuron™ Dopaminergic	$399 (EXGS-QNDSV)	$399 (EXGS-QND)	$169 (EXGS-QNDM)
	Quick-Neuron™ GABAergic	$399 (EXGS-QNGSV)	$399 (EXGS-QNG)	$149 (EXGS-QNGM)
	Quick-Muscle™ Skeletal	$349 (EXGS-QMSSV)		$129 (EXGS-QMSM)
	Quick-Hepatocyte™	$499 (EXGS-QHSV)		$149 (EXGS-QHM)
	Quick-miniBrain™	$999 (EXGS-QMBSVMR)		

15% off for 3 or more kit purchases per order

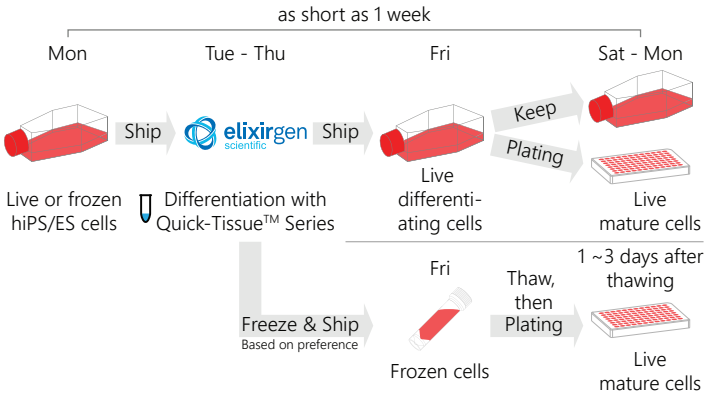
* This kit provides 2 wells per each tissue (total 6 wells of 24-well plate)

** This kit is for 48 wells of a 96-well microplate format

Category	Product	Size	Request Quote (Catalog number)
Quick-Tissue™ Series Differentiation Support	Quick-Tissue™ Mesendoderm Booster	8 wells of 24-well plate	$99 (EXGS-QTMB)
	Quick-Tissue™ Adaptation Kit	a 35-mm dish or 1 well of 6-well plate	$169 (EXGS-QTA1)
	New product coming soon!		
Reagents for Maintaining Undifferentiated Stem Cells	Ajinomoto StemFit® Basic02	500 mL	Ask (EXGS-ASB02)
	Nippi iMatrix-511 silk	175 µg x 6 tubes	Ask (EXGS-NI511S)
	Nippi iMatrix-511	175 µg x 6 tubes	$690 (EXGS-NI511)

15% off for 3 or more kit purchases per order

Quick-Tissue™ Stem Cell Differentiation Services



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 fronzen 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!