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TaqMan® hPSC Scorecard™ Panel

USER GUIDE

For rapid confirmation of pluripotency and prediction of differentiation potential

Catalog Numbers A15870, A15871, A15872, and A15876

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Product information

Kit contents and storage

Types of kits

This manual is supplied with the products listed below. For a list of components supplied with each catalog number, see below.

Product	Catalog no.
TaqMan® hPSC Scorecard™ Panel 384w	A15870
TaqMan® hPSC Scorecard™ Kit 384w	A15872
TaqMan® hPSC Scorecard™ Panel 2 × 96w FAST	A15876
TaqMan® hPSC Scorecard™ Kit 2 × 96w FAST	A15871

TaqMan® hPSC Scorecard™ 384w

The TaqMan® hPSC Scorecard™ Panel 384w (Cat. no. A15870) and the TaqMan® hPSC Scorecard™ Kit 384w (Cat. no. A15872) contain the components listed below. Each 384-well plate can be used to analyze four cDNA samples.

Component	Composition	Amount	Catalog no.	
			A15870	A15872
TaqMan® hPSC Scorecard™ Panel 384w	TaqMan® probes in a 384-well plate	1 plate	✓	✓
MicroAmp™ Optical Adhesive Film	Optical adhesive covers	1 each	✓	✓
TaqMan® Gene Expression Master Mix[1]	Solution containing AmpliTaq Gold™ DNA Polymerase UP (Ultra Pure), Uracil-DNA Glycosylase, dNTPs with dUTP, Passive Reference 1, and optimized mix components	5 mL		✓
TaqMan® hPSC Scorecard™ Panel QRC	TaqMan® hPSC Scorecard™ Panel Quick Reference Card	1 each	✓	✓

[1] TaqMan® Gene Expression Master Mix is also available separately under Cat. no. 4369016 (see "TaqMan® hPSC Scorecard™ panel products" on page 41).

TaqMan® hPSC Scorecard™ 2 × 96w FAST

The TaqMan® hPSC Scorecard™ Panel 2 × 96w FAST (Cat. no. A15876) and the TaqMan® hPSC Scorecard™ Kit 2 × 96w FAST (Cat. no. A15871) contain the components listed below. Each 96-well plate can be used to analyze one cDNA sample.

Component	Composition	Amount	Catalog no.	
			A15876	A15871
TaqMan® hPSC Scorecard™ Panel 96w FAST	TaqMan® probes in a 96-well plate	2 plates	✓	✓
MicroAmp™ Optical Adhesive Film	Optical Adhesive Covers	2 each	✓	✓
TaqMan® Fast Advanced™ Master Mix[1]	Solution containing: AmpliTaq™ Fast DNA Polymerase, Uracil-N glycosylase (UNG), dNTPs with dUTP, ROX™ dye (passive reference), and optimized buffer components	2 × 1 mL		✓
TaqMan® hPSC Scorecard™ Panel QRC	TaqMan® hPSC Scorecard™ Panel Quick Reference Card	1 each	✓	✓

[1] TaqMan® Fast Advanced™ Master Mix is also available separately under Cat. no. 4444556 (see "TaqMan® hPSC Scorecard™ panel products" on page 41).

Shipping and storage

The TaqMan® hPSC Scorecard™ Panels and Kits are shipped on wet ice. Upon receipt, transfer the entire shipment to 2–8℃ for immediate storage. Store the individual components as described below. The performance of the products is guaranteed for 2 months from the date of purchase, if stored and handled properly.

Component	Storage and Handling
TaqMan® hPSC Scorecard™ Panel 384w or 96w FAST plates	Store at 4℃–30℃. Maintain in foil bag until ready to for use. Briefly spin plates at 400 × *g* for 2 minutes prior to use.
MicroAmp™ Optical Adhesive Film	Store at 4℃–30℃. **Protect from dust.**
TaqMan® Gene Expression Master Mix	Store at 2℃–8℃. **DO NOT FREEZE.**
TaqMan® Fast Advanced™ Master Mix	Store at 2℃–8℃. **DO NOT FREEZE.**

Description of the system

TaqMan® hPSC Scorecard™ panels

TaqMan® hPSC Scorecard™ Panel 384w and TaqMan® hPSC Scorecard™ Panel 96w FAST are MicroAmp™ Optical 96- or 384-well Reaction Plates, standard or Fast, that contain dried-down TaqMan® Gene Expression Assays specifically formulated for evaluating human embryonic stem cells (ESC) and human induced pluripotent stem cells (iPSC) to confirm their pluripotency and to predict their differentiation potential and outcome. The gene expression assays contain a collection of predesigned, gene-specific primer and probe sets for performing quantitative gene expression studies on the cDNA samples prepared from undifferentiated or differentiated human ESCs and iPSCs. For more information on TaqMan® Gene Expression Assays, see "Appendix C, "Background information"").

How hPSC Scorecard™ panels work

After isolating total RNA from human ESC or iPSC cultures and using it to generate cDNA in a reverse transcription (RT) reaction, TaqMan® hPSC Scorecard™ Panels and associated reagents are used to quantitate RNA expression levels of genetic markers for pluripotency and differentiation potential, as well as endogenous controls. The gene expression data are then analyzed using the web-based hPSC Scorecard™ Analysis Software for the pluripotency and differentiation potential of the cells from which the total RNA is isolated.

To do this, you:

- Isolate total RNA from human ESCs or iPSCs by TRIzol™ organic phase extraction or other preferred method.
- Prepare each cDNA sample by performing eight reverse transcription reactions per total RNA sample.
- Combine the appropriate TaqMan® master mix with your cDNA sample and RNase-free water, and reconstitute each well of the TaqMan® hPSC Scorecard™ Panel by adding 10 µL of the reaction mixture per well.
- Load and run the plates on a compatible real-time PCR (RT-PCR) instrument using either standard or Fast thermal cycling conditions.
- Analyze the gene expression data using the web-based hPSC Scorecard™ Analysis Software to confirm the pluripotency of the samples and predict their differentiation potential and outcome. The hPSC Scorecard™ Analysis Software is available at **https://apps.thermofisher.com/hPSCscorecard/home.htm**.

Types of hPSC Scorecard™ panels

TaqMan® hPSC Scorecard™ Panels are available as 384-well plates (Cat. nos. A15870, A15872) or as 96-well FAST plates (Cat. nos. A15876, A15871) for use with Fast thermal cycling conditions.

- **TaqMan® hPSC Scorecard™ Panel 384w** are 384-well MicroAmp™ optical assay plates, which allow the analysis of four separate cDNA samples under standard thermal cycling conditions.
- **TaqMan® hPSC Scorecard™ Panel 96w FAST** are 96-well MicroAmp™ optical Fast thermal cycling plates, which reduce quantitative PCR run times to less than 40 minutes when used under Fast thermal cycling conditions in a compatible RT-PCR system. Each 96-well plate allows the analysis of one cDNA sample.

Compatible TaqMan® master mixes

Each well in a TaqMan® hPSC Scorecard™ Panel contains a pair of unlabeled PCR primers specific to a pluripotency or differentiation marker or endogenous control, and a TaqMan® probe with a fluorescent dye-label on the 5′ end (e.g., FAM™ or VIC™ dye) and a minor groove binder (MGB) and non-fluorescent quencher (NFQ) on the 3′ end (see Appendix C, "Background information").

The assays in each well are reconstituted to a 1✕ formulation using the compatible TaqMan® master mix as described in this user guide and are designed to run under standard or Fast cycling conditions for two-step RT-PCR.

The table below lists the TaqMan® hPSC Scorecard™ Panel and corresponding TaqMan® master mix compatible with it. Note that TaqMan® hPSC Scorecard™ Kits contain the appropriate compatible TaqMan® master mix, which are not supplied with the individually packaged TaqMan® hPSC Scorecard™ Panels and need to be purchased separately (see "TaqMan® hPSC Scorecard™ panel products" on page 41 for ordering information).

TaqMan® hPSC Scorecard™ Panel	Compatible TaqMan® master mix
TaqMan® hPSC Scorecard™ Panel 384w	TaqMan® Gene Expression Master Mix (for standard cycling)
TaqMan® hPSC Scorecard™ Panel 96w FAST	TaqMan® Fast Advanced™ Master Mix (for Fast cycling)

Compatible RT-PCR instruments

TaqMan® hPSC Scorecard™ Panels can be used with the Applied Biosystems™ RT-PCR systems listed below. Note that TaqMan® hPSC Scorecard™ Panel 96w FAST must be run on RT-PCR systems that contain Fast thermal cycling blocks. Alternately, TaqMan® hPSC Scorecard™ Panel 384w must be run on RT-PCR systems with standard thermal cycling blocks.

TaqMan® hPSC Scorecard™ Panel	Compatible Applied Biosystems™ RT-PCR systems
TaqMan® hPSC Scorecard™ Panel 384w	• QuantStudio™ 12K Flex System with 384-well Block • ViiA™ 7 Real-Time PCR System with 384-well Block
TaqMan® hPSC Scorecard™ Panel 96w FAST	• StepOnePlus™ Real-Time PCR System • ViiA™ 7 Real-Time PCR System with Fast 96-well Block

TaqMan® hPSC Scorecard™ panel workflow

Experiment outline

The table below describes the major steps needed to generate and prepare cDNA samples for analysis using the TaqMan® hPSC Scorecard™ Panels. For more details, refer to the pages indicated.

Step	Action	Page
1	Harvest ESCs or iPSCs from: Undifferentiated cells (feeder-dependent culture) Undifferentiated cells (feeder-free culture in StemPro™ hESC SFM™) Undifferentiated cells (feeder-free culture in Essential 8™ medium) Randomly differentiated cells (i.e., embryoid bodies)	"Undifferentiated cells (Feeder-Dependent culture) " on page 12 "Undifferentiated cells (Feeder-Free culture in StemPro™ hESC SFM™)" on page 15 "Undifferentiated cells (Feeder-Free culture in Essential 8™ medium)" on page 17 "Randomly differentiated cells (Embryoid bodies)" on page 20
2	Isolate total RNA from cells	"Total RNA isolation by TRIzol™ organic phase extraction (Recommended method)" on page 24
3	*Optional*: Remove contaminating DNA from RNA sample	"Optional: DNase treatment" on page 25
4	Quantitate total RNA and asses its quality	"RNA quantification and quality" on page 27
5	Generate cDNA by reverse transcription	Chapter 4, "cDNA preparation"
6	Perform TaqMan® qRT-PCR	Chapter 5, "TaqMan® qRT-PCR"
7	Analyze data using the hPSC Scorecard™ Analysis Software	"Analyze the results" on page 32

Sample generation

Undifferentiated cells (Feeder-Dependent culture)

Introduction

This section provides instructions on harvesting undifferentiated ESCs or iPSCs that are maintained as feeder-dependent cultures on inactived murine embryonic fibroblast (MEF) feeder cells for total RNA extraction. You will need to harvest at least 5×10^5 cells per sample to isolate sufficient total RNA for the subsequent steps of the workflow.

IMPORTANT! Feeder-dependent ESCs or iPSCs should be cultured feeder-free on Geltrex™-matrix coated culture vessels for one passage in MEF-conditioned medium before the cells are harvested and total RNA is isolated.

Materials needed

- DMEM/F-12 with GlutaMAX™-I (Cat. no. 10565-018)
- KnockOut™ Serum Replacement (KSR) (Cat. no. 10828-010)
- MEM™ Non-Essential™ Amino Acids Solution (10 mM) (Cat. no. 11140-050)
- β-Mercaptoethanol (1000X), liquid (Cat. no. 21985-023)
- Basic Fibroblast Growth Factor (bFGF), recombinant human (Cat. no. PGH0264)
- Collagenase, Type IV (Cat. no. 17104-019)
- Attachment Factor (Cat. no. S-006-100)
- DMEM with GlutaMAX™-I (high glucose) (Cat. no. 10569-010)
- Fetal Bovine Serum, ESC-Qualified (Cat. no. 16141-079)
- DPBS, no Calcium, no Magnesium (Cat. no. 14190-144)
- Geltrex™, hESC qualified (Cat. no. A1413302)
- TRIzol™ Reagent (Cat. no. 18596-018)
- Gibco™ Mouse Embryonic Fibroblasts (Irradiated) (Cat. no. S1520-100)
- Cell Scraper (Falcon™, Cat. no. 353085)
- Appropriate tissue culture plates and supplies

Prepare media, reagents, and matrix-coated culture vessels

The following media, reagents, and culture vessels are needed for culturing and harvesting undifferentiated, feeder-dependent ESCs or iPSCs.

For instructions on preparing the media and reagents listed below, see "Appendix A, "Recipes"". For instructions on coating culture vessels with Geltrex™ matrix, see "Appendix B, "Preparing culture vessels"".

- MEF medium
- ESC medium
- MEF-conditioned medium (MEF-CM)
- 1X Collagenase IV Solution (1 mg/mL)
- Geltrex™ matrix-coated culture vessels

Culture and harvest cells

1. Culture ESCs or iPSCs on inactivated MEF feeder cells using complete ESC medium.

2. When the ESC or iPSC culture has reached the desired confluency to yield at least 5×10^5 cells per sample, aspirate off the cell culture medium (i.e., ESC medium).

3. Add Collagenase Type IV (1 mg/mL) solution to the dish containing ESCs or iPSCs. Adjust the volume of Collagenase IV for various dish sizes (refer to Table 1.

Table 1 Volume of collagenase IV (1 mg/mL) and MEF-CM required

Culture Vessel	Surface Area	Volume of Collagenase IV	Volume of MEF-CM
6-well plate	10 cm^2/well	1.0 mL per well	2.0 mL per well
12-well plate	4 cm^2/well	0.5 mL per well	1.0 mL per well
24-well plate	2 cm^2/well	0.25 mL per well	0.5 mL per well
35-mm dish	10 cm^2	1.0 mL	2.0 mL
60-mm dish	20 cm^2	2.0 mL	4.0 mL
100-mm dish	60 cm^2	6.0 mL	10.0 mL

4. Incubate for 30–45 minutes in a 37℃, 5% CO_2 incubator.

   Note: Incubation times may vary among different batches of collagenase; therefore, you need to determine the appropriate incubation time by examining the colonies. Note that the required exposure time shortens as the enzymatic activity of collagenase increases (i.e., 200,000 U/mg at 1 mg/mL concentration will take longer than stocks of 290,000 U/mg at 1 mg/mL concentration).

5. When the edges of the colonies are starting to pull away from the vessel, carefully aspirate the Collagenase IV solution from the vessel without disturbing the attached cell layer and add 3 mL of ESC medium.

6. Dislodge the cells with a 5-mL pipet by gently blowing the cells off the surface of the vessel while pipetting up and down. This will not only dislodge the colonies, but also manually break them up into small fragments to be passaged. Do not create a single cell suspension; usually 5–10 pipetting motions will suffice to dislodge and resuspend the cells.

7. Transfer the cell clumps into a 15-mL tube. Use an additional 2 mL of medium to collect any cell clumps remaining in the vessel and add to the 15-mL conical tube.

8. Allow the cells to gravity sediment for approximately 5 minutes. This will permit the larger clumps to pellet out while allowing the removal of smaller clumps, single cells, and any iMEFs from the feeder layer that were dislodged through the process and still floating in the supernatant.

9. Aspirate the supernatant, and then gently tap the tube to loosen the cell pellet from the bottom of the tube.

10. Add the appropriate amount of ESC medium for your passaging split ratio. At this point you can continue to gently dissociate the cells into small clusters (50–500 cells) by gentle pipetting. Avoid making single cell suspensions.

11. Seed the cell clusters onto Geltrex™-matrix coated culture vessel containing MEF-conditioned medium (MEF-CM). The final volume of the MEF-CM depends on the vessel used (refer to Table 2, for the final volume of medium required for each vessel). Use a typical split ratio of 1:3 to 1:5 per plate, depending on amount of cell clusters.

12. Move the culture vessel in several quick, short, back-and-forth and side-to-side motions to disperse the cells evenly across its surface, and then return the vessel to the incubator.

13. Continue to culture the cells until they are 80–90% confluent, changing the spent MEF-CM daily.

14. On the day of harvesting, aspirate the culture medium and wash the cells once with 5 mL of DPBS for 2–3 minutes.

15. Aspirate the DPBS and discard.

16. Add 1 mL of TRIzol™ reagent and incubate for 2–3 minutes. Scrape the plate with a sterile cell scraper and collect the slurry into a sterile, RNAse-free microcentrifuge tube.

17. Store the cells at –80℃ until ready for RNA isolation.

Undifferentiated cells (Feeder-Free culture in StemPro™ hESC SFM™)

Introduction

This section provides instructions on harvesting undifferentiated ESCs or iPSCs maintained as feeder-free cultures on Geltrex™ matrix-coated vessels in complete StemPro™ hESC medium for total RNA extraction. You will need to harvest at least 5×10^5 cells per sample to isolate sufficient total RNA for the subsequent steps of the workflow.

Materials needed

- DMEM/F-12 with GlutaMAX™-I (Cat. no. 10565-018)
- StemPro™ hESC SFM™ Kit (Cat. no. A1000701)
- β-Mercaptoethanol (1000X), liquid (Cat. no. 21985-023)
- Basic Fibroblast Growth Factor (bFGF), recombinant human (Cat. no. PGH0264)
- Collagenase, Type IV (Cat. no. 17104-019)
- DPBS, no Calcium, no Magnesium (Cat. no. 14190-144)
- Geltrex™, hESC qualified (Cat. no. A1413302)
- TRIzol™ Reagent (Cat. no. 18596-018)
- Cell Scraper (Falcon™, Cat. no. 353085)
- Appropriate tissue culture plates and supplies

Prepare media, reagents, and matrix-coated culture vessels

The following media, reagents, and culture vessels are needed for culturing and harvesting undifferentiated ESCs or iPSCs maintained as feeder-free cultures in complete StemPro™ hESC medium.

For instructions on preparing the media and reagents listed below, see “Appendix A, “Recipes””. For instructions on coating culture vessels with Geltrex™ matrix, see “Appendix B, “Preparing culture vessels””.

- StemPro™ hESC SFM™ medium
- StemPro™ wash solution
- 10X Collagenase IV Solution (10 mg/mL)
- Geltrex™ matrix-coated culture vessels

Culture and harvest cells

1. Coat the culture vessels with Geltrex™ matrix at least 1 hour before passaging the cells. Warm the appropriate amount of 10 mg/mL (10X) Collagenase IV solution, StemPro™ wash solution, and complete StemPro™ hESC SFM™ medium to 37°C in a water bath.

2. When the ESC or iPSC culture has reached the desired confluency to yield at least 5×10^5 cells per sample, aspirate off the cell culture medium (i.e., StemPro™ hESC SFM™).

3. Gently add 10 mg/mL Collagenase Type IV (10X) solution to the culture vessel containing ESCs or iPSCs. Adjust the volume of Collagenase IV for various dish sizes (refer to Table 2).

Table 2 Volume of collagenase IV (10 mg/mL) and complete StemPro™ hESC SFM™ medium required

Culture Vessel	Surface Area	Volume of Collagenase IV	Volume of complete StemPro™ hESC SFM™
6-well plate	10 cm^2/well	1.0 mL per well	2.0 mL per well
12-well plate	4 cm^2/well	0.5 mL per well	1.0 mL per well
24-well plate	2 cm^2/well	0.25 mL per well	0.5 mL per well
35-mm dish	10 cm^2	1.0 mL	2.0 mL
60-mm dish	20 cm^2	2.0 mL	4.0 mL
100-mm dish	60 cm^2	6.0 mL	10.0 mL

4. Incubate for 3–5 minutes in a 37℃, 5% CO_2 incubator until the edges of the colonies begin to curl.

   Note: Incubation times may vary among different batches of collagenase; therefore, you need to determine the appropriate incubation time by examining the colonies. Note that the required exposure time shortens as the enzymatic activity of collagenase increases (i.e., 200,000 U/mg at 1 mg/mL concentration will take longer than stocks of 290,000 U/mg at 1 mg/mL concentration).

5. When the edges of the colonies are starting to pull away from the vessel, carefully aspirate the Collagenase IV solution from the vessel without disturbing the attached cell layer and gently rinse the cells with 3 mL of pre-warmed StemPro™ wash solution.

6. Aspirate the wash solution and add the appropriate volume of pre-warmed complete StemPro™ hESC SFM™ medium (refer to Table 2).

7. Use a sterile cell scraper to gently remove the cell clumps. Pipette the cell suspension across the plate surface with a 5-mL pipette to break up the detached colonies into smaller clumps. Do not create a single cell suspension; usually 3–5 pipetting motions will suffice to dislodge and resuspend the cells.

8. Gently transfer the cell clumps into a 15-mL tube. Depending on the culture vessel, use an additional 1–3 mL of pre-warmed complete StemPro™ hESC SFM™ medium to collect any cell clumps remaining in the vessel and add to the 15-mL conical tube.

9. Centrifuge cell suspension at 200 × *g* for 2 minutes or allow the cells to gravity sediment at room temperature for 5–10 minutes.

10. Aspirate off the Geltrex™ solution from the fresh Geltrex™-matrix coated vessel and add the appropriate volume of pre-warmed complete StemPro™ hESC SFM™ medium (refer to Table 2).

11. Following centrifugation or gravity sedimentation of the cell suspension (step 9), aspirate off the supernatant and gently re-suspend the cell clumps with the appropriate amount of complete StemPro™ hESC SFM™ medium.

12. Add the desired amount of cell suspension into each new Geltrex™-matrix coated vessel according to the desired split ratio.

    Note: The split ratio is variable, though generally between 1:4 and 1:6. If the cells are overly dense and crowding, increase the ratio, and if the cells are sparse, decrease the ratio. Cells will need to be split every 4–6 days based upon their appearance.

13. Move the culture vessel in several quick, short, back-and-forth and side-to-side motions to disperse the cells evenly across its surface, and then return the vessel to the incubator.

14. The next day, gently aspirate the medium to remove the non-attached cells, and replace it with fresh complete StemPro™ hESC SFM™ medium. Replace the spent medium with fresh medium every day thereafter.

15. Continue to culture the cells until they are 80–90% confluent, changing the medium daily.

16. On the day of harvesting, aspirate the culture medium and wash the cells once with 5 mL of DPBS for 2–3 minutes.

17. Aspirate the DPBS and discard.

18. Add 1 mL of TRIzol™ reagent and incubate for 2–3 minutes. Scrape the plate with a sterile cell scraper and collect the slurry into a sterile, RNAse-free microcentrifuge tube.

19. Store the cells at –80℃ until ready for RNA isolation.

Undifferentiated cells (Feeder-Free culture in Essential 8™ medium)

Introduction

This section provides instructions on harvesting undifferentiated ESCs or iPSCs maintained as feeder-free cultures on Geltrex™ matrix-coated vessels in complete Essential 8™ medium for total RNA extraction. You will need to harvest at least 5×10^5 cells per sample to isolate sufficient total RNA for the subsequent steps of the workflow.

Materials needed

- Essential 8™ Medium (prototype) (Cat. no. A14666SA)
- DMEM/F-12 with GlutaMAX™-I (Cat. no. 10565-018)
- Collagenase, Type IV (Cat. no. 17104-019)
- DPBS, no Calcium, no Magnesium (Cat. no. 14190-144)
- Geltrex™, hESC qualified (Cat. no. A1413302)
- TRIzol™ Reagent (Cat. no. 18596-018)
- Cell Scraper (Falcon™, Cat. no. 353085)
- Appropriate tissue culture plates and supplies

Prepare media, reagents, and matrix-coated culture vessels

The following media, reagents, and culture vessels are needed for culturing and harvesting undifferentiated ESCs or iPSCs maintained as feeder-free cultures on in complete Essential 8™ medium.

For instructions on preparing the media and reagents listed below, see "Appendix A, "Recipes"". For instructions on coating culture vessels with Geltrex™ matrix, see "Appendix B, "Preparing culture vessels"".

- Essential 8™ Medium
- 10X Collagenase IV Solution (10 mg/mL)
- Geltrex™ matrix-coated culture vessels

Culture and harvest cells

1. Coat the culture vessels with Geltrex™ matrix at least 1 hour before passaging the cells. Warm the appropriate amount of 10 mg/mL (10X) Collagenase IV solution and DMEM/F-12 to 37°C in a water bath.

2. Warm complete Essential 8™ medium required for that day at room temperature until it is no longer cool to the touch, approximately 20–30 minutes. **Do not warm the Essential 8™ medium at 37°C.**

3. When the ESC or iPSC culture has reached the desired confluency to yield at least 5×10^5 cells per sample, aspirate off the cell culture medium (i.e., Essential 8™ medium).

4. Gently add 10 mg/mL Collagenase Type IV (10X) solution to the culture vessel containing ESCs or iPSCs. Adjust the volume of Collagenase IV for various dish sizes (refer to Table 3).

Table 3 Volume of collagenase IV (10 mg/mL) and complete Essential 8™ medium required

Culture Vessel	Surface Area	Volume of Collagenase IV	Volume of complete Essential 8™ medium
6-well plate	10 cm^2/well	1.0 mL per well	2.0 mL per well
12-well plate	4 cm^2/well	0.5 mL per well	1.0 mL per well
24-well plate	2 cm^2/well	0.25 mL per well	0.5 mL per well
35-mm dish	10 cm^2	1.0 mL	2.0 mL
60-mm dish	20 cm^2	2.0 mL	4.0 mL
100-mm dish	60 cm^2	6.0 mL	10.0 mL

5. Incubate for 3–5 minutes in a 37°C, 5% CO_2 incubator until the edges of the colonies begin to curl.

   Note: Incubation times may vary among different batches of collagenase; therefore, you need to determine the appropriate incubation time by examining the colonies. Note that the required exposure time shortens as the enzymatic activity of collagenase increases (i.e., 200,000 U/mg at 1 mg/mL concentration will take longer than stocks of 290,000 U/mg at 1 mg/mL concentration).

6. When the edges of the colonies are starting to pull away from the vessel, carefully aspirate the Collagenase IV solution from the vessel without disturbing the attached cell layer and gently rinse the cells with 3 mL of pre-warmed DMEM/F-12 solution.

7. Aspirate the wash solution and add the appropriate volume of pre-warmed complete Essential 8™ medium (refer to Table 3).

8. Use a sterile cell scraper to gently remove the cell clumps. Pipette the cell suspension across the plate surface with a 5-mL pipette to break up the detached colonies into smaller clumps. Do not create a single cell suspension; usually 3–5 pipetting motions will suffice to dislodge and resuspend the cells.

9. Gently transfer the cell clumps into a 15-mL tube. Depending on the culture vessel, use an additional 1–3 mL of pre-warmed complete StemPro™ hESC SFM™ medium to collect any cell clumps remaining in the vessel and add to the 15-mL conical tube.

10. Centrifuge cell suspension at 200 × *g* for 2 minutes or allow the cells to gravity sediment at room temperature for 5–10 minutes.

11. Aspirate off the Geltrex™ solution from the fresh Geltrex™-matrix coated vessel and add the appropriate volume of pre-warmed complete Essential 8™ medium (refer to Table 3).

12. Following centrifugation or gravity sedimentation of the cell suspension (step 10), aspirate off the supernatant and gently re-suspend the cell clumps with the appropriate amount of complete Essential 8™ medium.

13. Add the desired amount of cell suspension into each new Geltrex™-matrix coated vessel according to the desired split ratio.

    Note: The split ratio is variable, though generally between 1:4 and 1:6. If the cells are overly dense and crowding, increase the ratio, and if the cells are sparse, decrease the ratio. Cells will need to be split every 4–6 days based upon their appearance.

14. Move the culture vessel in several quick, short, back-and-forth and side-to-side motions to disperse the cells evenly across its surface, and then return the vessel to the incubator.

15. The next day, gently aspirate the medium to remove the non-attached cells, and replace it with fresh complete Essential 8™ medium. Replace the spent medium with fresh medium every day thereafter.

16. Continue to culture the cells until they are 80–90% confluent, changing the medium daily.

17. On the day of harvesting, aspirate the culture medium and wash the cells once with 5 mL of DPBS for 2–3 minutes.

18. Aspirate the DPBS and discard.

19. Add 1 mL of TRIzol™ reagent and incubate for 2–3 minutes. Scrape the plate with a sterile cell scraper and collect the slurry into a sterile, RNAse-free microcentrifuge tube.

20. Store the cells at –80°C until ready for RNA isolation.

Randomly differentiated cells (Embryoid bodies)

Introduction

This section provides instructions on generating embryoid bodies (EBs) for random differentiation from ESCs or iPSCs maintained as feeder-dependent or feeder-free cultures, and their subsequent harvest for total RNA extraction.

IMPORTANT! There are several methods for creating EBs for random differentiation. Here we cover suspension EB culture, which is recommended for a maximum of 7 days. If extended EB culture is desired, we recommend seeding the EBs on a Geltrex™-matrix coated culture vessel and continuing as an adherent culture as described in ““Harvest cells (Option 2: 4 day EB suspension and adherent culture)“ on page 22”, until total RNA isolation.

Materials needed

- DMEM/F-12 with GlutaMAX™-I (Cat. no. 10565-018)
- KnockOut™ Serum Replacement (KSR) (Cat. no. 10828-010)
- MEM™ Non-Essential™ Amino Acids Solution (10 mM) (Cat. no. 11140-050)
- β-Mercaptoethanol (1000X), liquid (Cat. no. 21985-023)
- DPBS, no Calcium, no Magnesium (Cat. no. 14190-144)
- TRIzol™ Reagent (Cat. no. 18596-018)
- Cell Scraper (Falcon™, Cat. no. 353085)
- 60-mm Petri dish (non-tissue culture treated) or ultra-low binding dish
- Appropriate tissue culture plates and supplies
- Geltrex™, hESC qualified (Cat. no. A1413302; for optional adherent EB culture)

Prepare media and matrix-coated culture vessels

The following media and matrix-coated culture vessels are needed for creating and harvesting EBs for total RNA isolation.

For instructions on preparing the media listed below, see “Appendix A, “Recipes“”. For instructions on coating culture vessels with Geltrex™ matrix, see “Appendix B, “Preparing culture vessels“”.

- EB medium
- Geltrex™ matrix-coated culture vessels (for optional adherent EB culture)

Generate EBs from ESCs/iPSCs

Embryoid Bodies (EBs) are generated at a normally scheduled passage by plating ESCs or iPSCs into non-tissue culture-treated dishes to prevent attachment and allowing them to aggregate to form EBs.

1. Culture ESCs or iPSCs on MEF feeder cells or in the desired feeder-free condition (StemPro™ hESC SFM™, MEF-CM, or Essential 8™ Medium) until the cells are approximately 90% confluent.

2. Aspirate off the culture medium from the culture plates or dishes, and then add 1 mL pre-warmed EB medium to each well of 6-well plate or to each 35-mm dish, 2 mL to each 60-mm dish, or 3 mL to each 100-mm dish.

3. Roll the StemPro™ EZPassage™ disposable stem cell passaging tool across the entire dish or plate in one direction (left to right). Rotate the culture dish or plate 90 degree, and roll the StemPro™ EZPassage™ disposable stem cell passaging tool across the entire dish or plate.

4. Use a cell scraper to gently detach the cells off the surface of the culture vessel.

5. Gently transfer the cell clumps using a 5-mL pipette into a 15-mL conical tube. **Do not break the cell clumps into small pieces.**

6. Depending on the culture vessel, use an additional 1–3 mL of pre-warmed EB medium (see step 2 on page 21) to collect any cell clumps remaining in the vessel and add to the 15-mL conical tube.

7. Transfer the cell clumps to a 60-mm Petri dish (non-tissue culture treated) or ultra-low binding dish in a total of 5 mL of EB medium. If necessary, add more EB medium into the 15-mL tube to bring up the volume to 5 mL.

   Note: If using a different size vessel, add more pre-warmed EB medium into the 15-mL tube to bring up the volume of the cell suspension to the appropriate level for the vessel used (refer to Table 4). Generally use one plate of ESCs or iPSCs per one plate of EBs to be formed.

   Table 4 volume of EB medium required

Culture Vessel	Surface Area	Final volume of cell suspension in EB medium
6-well plate	10 cm^2/well	2.0 mL per well
12-well plate	4 cm^2/well	1.0 mL per well
24-well plate	2 cm^2/well	0.5 mL per well
35-mm dish	10 cm^2	2.0 mL
60-mm dish	20 cm^2	5.0 mL
100-mm dish	60 cm^2	12.0 mL

8. Place Petri dish containing the cells in a 37℃, 5% CO_2 incubator to allow the cells to form EBs.

9. The next day and every other day thereafter, replace the spent medium with fresh, pre-warmed EB medium. To replace the medium, gently transfer the EB suspension into a 15-mL conical tube and allow the EBs to sediment down by gravity for 10–15 minutes. Then gently aspirate off the supernatant and re-suspend the EBs with fresh, pre-warmed EB medium. Return the EB suspension into the same culture vessel for continued growth.

10. On day 7 of incubation, harvest the cells as described in ““Harvest cells (Option 1: 7 day EB suspension)“ on page 22”.

    Note: Culturing EBs in suspension for 7 days is usually sufficient for the analysis of random differentiation. If extended culture is desired, use the optional protocol for adherent EB culture as described in ““Harvest cells (Option 2: 4 day EB suspension and adherent culture)“ on page 22”.

Harvest cells (Option 1: 7 day EB suspension)

1. On day 7 of EB suspension, gently transfer the cells and the medium from the Petri dish into a 15-mL conical tube. Use an additional 5 mL of DPBS to collect any remaining EBs from the culture dish and add into the conical tube.

2. Allow the EBs to sediment down by gravity for 10–15 minutes, and then aspirate off the supernatant (i.e., spent EB medium).

3. Using a P1000 pipettor, add 1 mL of TRIzol™ reagent and pipette up and down to assist in properly breaking up the cell clumps.

4. Incubate the EBs for 2–3 minutes. Repeat pipetting and incubation, if the EBs require more time to be lysed. Collect the slurry into a sterile RNAse-free microcentrifuge tube. Store at –80℃ until ready for RNA isolation.

Harvest cells (Option 2: 4 day EB suspension and adherent culture)

1. On Day 4, gently transfer the cells and the medium from the Petri dish into a 15-mL conical tube. Use an additional 5 mL of DPBS to collect any remaining EBs from the culture dish and add into the conical tube.

2. Allow the EBs to sediment down by gravity for 10–15 minutes in the cell culture hood.

3. Aspirate the supernatant (i.e., spent EB medium) and replace it with 5 mL of fresh EB medium.

4. Transfer the cells to a fresh 60-mm tissue culture-treated dish coated with Geltrex™-matrix. If using a different size culture dish, refer to Table 4 for the volume EB of medium needed. Place the dish containing the cells in the 37℃, 5% CO_2 incubator and change the spent medium every other day.

5. Allow the EBs to attach and the contents of the EBs to grow out from the EBs to obtain adherent cell types.

6. On Day 7 and 14 of total differentiation (or as desired), aspirate off the EB medium and wash the cells once with 5 mL of DPBS for 2–3 minutes.

7. Aspirate off the DPBS wash.

8. Add 1 mL of TRIzol™ reagent and incubate for 2–3 minutes. Scrape the plate with a sterile cell scraper and collect the slurry into an RNAse-free microcentrifuge tube. Store at –80℃ until ready for RNA isolation.

3 Sample preparation

Total RNA isolation by TRIzol™ organic phase extraction (Recommended method)

Introduction

This section provides instructions on extracting total RNA from the ESCs and iPSCs by TRIzol™ organic phase extraction to use as a template for synthesis of single-stranded cDNA. You will need at least 5×10^5 cells harvested per sample to isolate sufficient total RNA for the reverse transcription reaction.

Note: For optimal performance, we recommend isolating total RNA by organic phase extraction using the TRIzol™ reagent. Note that column based purification methods (see "Total RNA isolation using the TRIzol™ plus RNA purification kit (Alternative PureLink™ Column-Based method)" on page 26) may also yield high-quality RNA.

Materials needed

- TRIzol™ reagent (Cat. no. 15596-026)
- Chloroform (Sigma, Cat. no. C-2432)
- Isopropanol (Sigma, Cat. no. I9516)
- Ethanol (Sigma, Cat. no. E7023)
- UltraPure™ DNase/RNase-Free Distilled Water (Cat. no. 10977)

Isolate total RNA by TRIzol™ organic phase extraction

1. Incubate the lysate with TRIzol™ reagent (from the last step of the harvesting procedure) at room temperature for 5 minutes to allow complete dissociation of nucleoprotein complexes.

2. To the cells in TRIzol™ reagent add 0.2 mL of chloroform per 1 mL of TRIzol™ reagent, and shake the tube vigorously by hand for 15 seconds.

3. Incubate the sample at room temperature for 2–3 minutes and centrifuge at 12,000 × *g* for 15 minutes at 4℃.

   Note: The mixture separates into a lower red phenol-chloroform phase, an interphase, and a colorless upper aqueous phase. RNA remains exclusively in the aqueous phase. The upper aqueous phase is ~50% of the total volume.

4. Carefully remove the upper aqueous phase and transfer to a new tube. Avoid drawing any of the interphase or organic layer into the pipette when removing the aqueous phase.

5. Add 0.5 mL of 100% isopropanol to the aqueous phase per 1 mL of TRIzol™ reagent, and incubate at room temperature for 10 minutes.

6. Centrifuge at 12,000 × *g* for 10 minutes at 4℃.

7. Carefully remove the supernatant from the RNA pellet and wash with 1 ml 75% ethanol.

   Note: The RNA is often invisible prior to centrifugation, and forms a gel-like pellet on the side and at the bottom of the tube upon centrifugation.

8. Centrifuge the tube at 7500 × *g* for 5 minutes at 4℃.

9. Discard the supernatant and air dry the RNA pellet for 5–10 minutes.

10. Resuspend the RNA pellet in 20–50 µL RNase-free water.

Optional: DNase treatment

Introduction

One key variable to the success of any RT-PCR experiment is the quality of the template RNA. DNA removal is critical for ensuring high-quality RNA, because DNA can serve as a template during the PCR portion of the experiment, resulting in false positives, background, etc. Ideally, the total RNA sample should have less than 0.005% of genomic DNA by weight. We recommend treating the isolated total RNA with the DNA-*free*™ Kit, which digests the contaminating DNA to levels below the limit of detection by routine PCR.

Materials needed

- DNA-*free*™ Kit (Cat. no. AM1906; contains rDNase I, 10X DNase I Buffer, DNase Inactivation Reagent, and nuclease–free water)

Guidelines for using the DNA-*free*™ kit

- We recommend conducting the reactions in 0.5 mL tubes to facilitate removal of the supernatant after treatment with the DNase Inactivation Reagent.
- DNA-*free*™ reactions can be conducted in 96-well plates. We recommend using V-bottom plates because their shape makes it easier to remove the RNA from the pelleted DNase Inactivation Reagent at the end of the procedure.
- The recommended reaction size is 10–100 µL. A typical reaction is 50 µL.
- Routine DNase treatment removes 2 µg of genomic DNA from 50 µL reaction with ≤ 200 µg/mL nucleic acid; refer to product insert if more rigorous DNase treatment is needed.

DNA-*free*™ kit procedure

1. For a 50-µL reaction, combine the following reagents in a clean, DNase- and RNase-free 0.5-mL microcentrifuge tube, and mix gently.

RNA Sample	1–10 µg
10X DNase I Reaction Buffer	5 µL
rDNase I (2 Units)	1 µL
DEPC-treated water to bring reaction to 50 µL	X µL
Total	**50 µL**

2. Incubate the tube at 37℃ for 20–30 minutes.

3. Resuspend the DNase Inactivation Reagent by flicking or vortexing the tube, add 5 µL (0.1 volume) of the resuspended inactivation reagent to the reaction mix, and mix well.
4. Incubate for 2 minutes at room temperature, mixing the reaction occasionally.
5. Centrifuge at 10,000 × *g* for 1.5 minutes and transfer the RNA to a fresh tube.
6. RNA is now ready for reverse transcription.

Total RNA isolation using the TRIzol™ plus RNA purification kit (Alternative PureLink™ Column-Based method)

Introduction

The TRIzol™ PLUS™ RNA Purification Kit provides a simple, reliable, and rapid method for isolating high-quality total RNA from a wide variety of samples, including animal and plant cells and tissue, bacteria, and yeast. The kit utilizes the strong lysis capability of TRIzol™ reagent, followed by a convenient and time-saving silica-cartridge purification protocol from the PureLink™ RNA Mini Kit, to purify total RNA.

Materials needed

- TRIzol™ PLUS™ RNA Purification kit (Cat. no. 12183-555)
- Chloroform (Sigma, Cat. no. C-2432)
- Ethanol (Sigma, Cat. no. E7023)
- UltraPure™ DNase/RNase-Free Distilled Water (Cat. no. 10977)

Isolate total RNA using the TRIzol™ plus RNA purification kit

1. Incubate the lysate with TRIzol™ reagent (from the last step of the harvesting procedure) at room temperature for 5 minutes to allow complete dissociation of nucleoprotein complexes.
2. To the cells in TRIzol™ reagent add 0.2 mL of chloroform per 1 mL of TRIzol™ reagent, and shake the tube vigorously by hand for 15 seconds.
3. Incubate the sample at room temperature for 2–3 minutes and centrifuge at 12,000 × *g* for 15 minutes at 4℃.
4. Transfer ~600 µL of the colorless, upper phase containing the RNA to a fresh RNase-free tube and add an equal volume of 100% ethanol to obtain a final ethanol concentration of 50%. Mix well by vortexing.
5. Invert the tube to disperse any visible precipitate that may form after adding ethanol. Proceed to next step using the column.
6. Transfer up to 700 µL of sample to a Spin Cartridge (with a Collection Tube) and centrifuge at 12,000 × *g* for 15 seconds at room temperature. Discard the flow–through and reinsert the Spin Cartridge into the same Collection Tube.
7. Repeat above two steps until the entire sample has been processed.

8. *Optional*: If your downstream application requires DNA-*free*™ total RNA, proceed to "On-Column PureLink™ DNase Treatment during RNA Purification" at this time (for details, see the PureLink™ RNA Mini Kit user guide, available from **thermofisher.com**).
9. Add 700 µL Wash Buffer I to the Spin Cartridge. Centrifuge at 12,000 × *g* for 15 seconds at room temperature.
10. Discard the flow-through and the Collection Tube. Insert the Spin Cartridge into a new Collection Tube.
11. Add 500 µL Wash Buffer II with ethanol to the Spin Cartridge.
12. Centrifuge at 12,000 × *g* for 15 seconds at room temperature. Discard the flow-through, and reinsert the Spin Cartridge into the same Collection Tube.
13. Repeat Steps 5–6 once.
14. Centrifuge the Spin Cartridge and Collection Tube at 12,000 × *g* for 1 minute at room temperature to dry the membrane with attached RNA. Discard the Collection Tube and insert the Spin Cartridge into a Recovery™ Tube.
15. Add 30–100 µL RNase-Free Water to the center of the Spin Cartridge. Incubate at room temperature for 1 minute.
16. Centrifuge the Spin Cartridge with the Recovery™ Tube for 2 minutes at ≥ 12,000 × *g* at room temperature. The recovery tube contains the purified total RNA.

RNA quantification and quality

Introduction

We recommend using total RNA that is:

- Between 0.002 and 0.2 µg/µL
- Less than 0.005% of genomic DNA by weight
- Dissolved in a PCR-compatible buffer
- Free of RNase activity
- Free of inhibitors of reverse transcription and PCR
- Nondenatured

IMPORTANT! Denaturation of the RNA is not necessary and may reduce the yield of cDNA for some gene targets.

Asses total RNA amount and quality

- Use NanoDrop™ to quantify extracted RNA sample. Quality of RNA is best assessed using $A_{260/280}$, with the recommended value close to 2.0.
- RNA integrity can be further assessed by running the samples on a 1% Agarose gel and assessing the 2:1 ratio of the 28s and 18s RNA bands and the absence of degraded RNA that appears as small molecular weight smear.
- If using Bioanalyzer™, a RIN (RNA integrity number) value of higher than 5 maybe sufficient, but higher than 8 is ideal for downstream applications.

4 cDNA preparation

Reverse transcription of total RNA

Introduction

This section provides instructions on generating single-stranded cDNA from the total RNA by reverse transcription (RT) using the High-capacity cDNA Reverse Transcription kit with RNase Inhibitor.

Materials needed

- High-capacity cDNA Reverse Transcription Kit with RNase Inhibitor (Cat. no. 4374966)

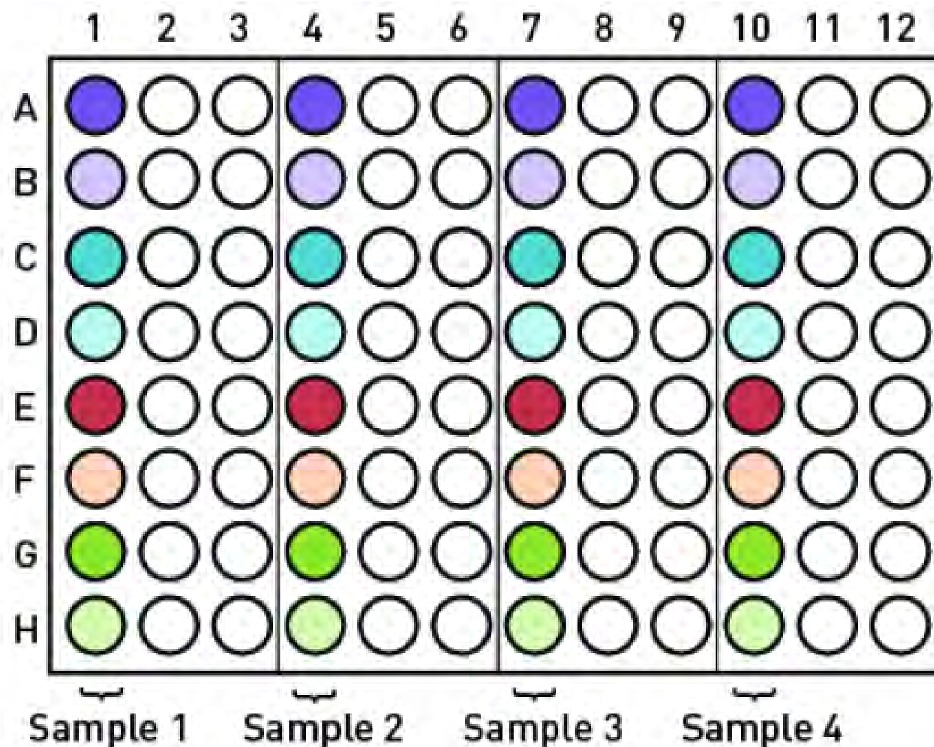
Perform RT reaction

1. Allow the components of the High-capacity cDNA Reverse Transcription Kit with RNase Inhibitor to thaw on ice
2. Prepare 2X RT master mix by mixing the following components:

Component	Per well	1 sample (8 wells)	4 samples (8 wells/sample)
10X TaqMan® RT Buffer	5 µL	50 µL	190 µL
25X dNTP Mix	2 µL	20 µL	76 µL
10X Random Primers	5 µL	50 µL	190 µL
MultiScribe™ Reverse Transcriptase (50 U/µL)	2.5 µL	25 µL	95 µL
RNase Inhibitor (20 U/ µL)	2.5 µL	25 µL	95 µL
RNase-free water	8 µL	80 µL	304 µL
TOTAL	25 µL	250 µL	950 µL

3. Place the 2X RT master mix on ice and mix gently.
4. Prepare RNA samples by diluting 1 µg total RNA in a total of 225 µL of RNase-free water.
5. Add 225 µL of 2X RT master mix to the diluted RNA and mix well.

6. Aliquot 50 µL of the above RNA plus RT mix in 8 vertical wells of a 96-well plate or an 8-strip PCR tube (see image).



7. Run the RT reaction in a thermal cycler using conditions as listed below.

Step	Temperature	Time
1	25℃	10 minutes
2	37℃	120 minutes
3	85℃	5 minutes
4	4℃	hold

8. Proceed to TaqMan® qRT-PCR, . If you do not proceed immediately to PCR amplification, store all cDNA samples at −15℃ to −25℃. To minimize freeze-thaw cycles, store the cDNA in smaller aliquots.

TaqMan® qRT-PCR

qRT-PCR using the TaqMan® hPSC Scorecard™ panel

Introduction

This section provides instructions on analyzing your cDNA samples by qRT-PCR using the TaqMan® hPSC Scorecard™ Panel.

Materials needed

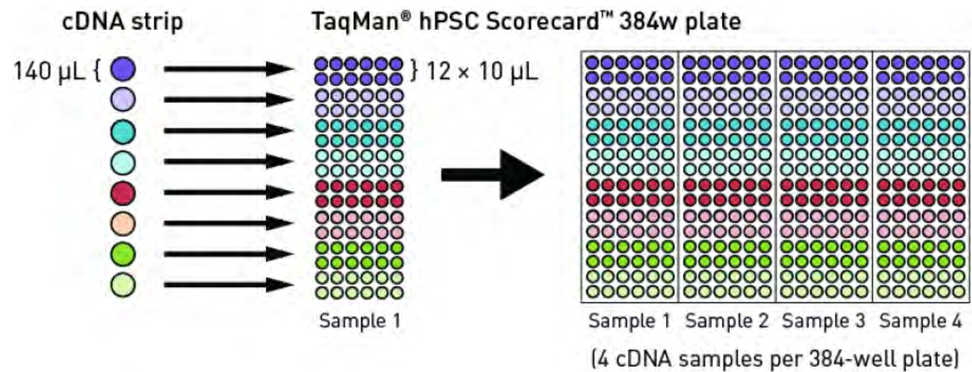
- TaqMan® hPSC Scorecard™ Panel (TaqMan® hPSC Scorecard™ Panel 384w or TaqMan® hPSC Scorecard™ Panel 96w FAST)
- TaqMan® Fast Advanced™ Master Mix (96-well format for running in FAST mode using the TaqMan® hPSC Scorecard™ Panel 96w FAST)
- TaqMan® Gene Expression Master Mix (384-well format using the TaqMan® hPSC Scorecard™ Panel 384w)
- MicroAmp™ Optical Adhesive Film

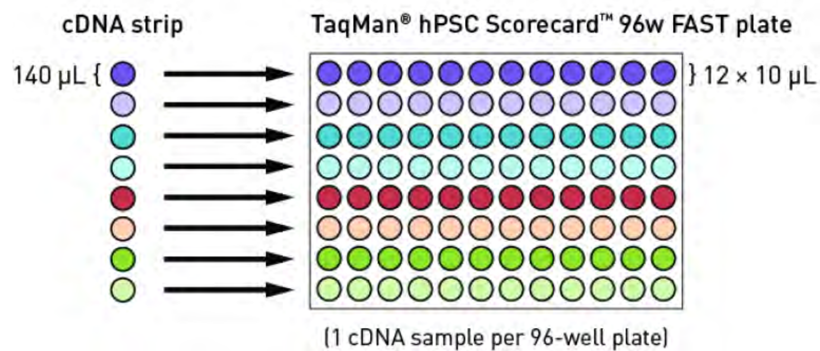
Run the qRT-PCR

1. Dilute each well containing 50 µL cDNA with 20 µL PCR water for a final volume of 70 µL.

2. Add 70 µL 2X TaqMan® Gene Expression Master Mix (if using the TaqMan® hPSC Scorecard™ Panel 384w) or 70 µL 2X TaqMan® Fast Advanced™ Master Mix (if using the TaqMan® hPSC Scorecard™ Panel 96w FAST).

3. Load 10 µL per well using multichannel pipette onto the 384-well or the 96-well plate using fresh tips each time as shown below. For 96-well plates, one well is sufficient to load one row of the plate.





4. Seal the plate with the MicroAmp™ Optical Adhesive Film, and centrifuge it at 600 × *g* for 2 minutes.

5. Place the plate in a compatible RT-PCR instrument equipped with the appropriate thermal block.

IMPORTANT! TaqMan® hPSC Scorecard™ Panel 96w FAST must be run on RT-PCR systems that contain Fast thermal cycling blocks and the TaqMan® hPSC Scorecard™ Panel 384w must be run on systems with standard thermal cycling blocks. For a list of Applied Biosystems™ RT-PCR systems compatible with TaqMan® hPSC Scorecard™ Panels, see "Compatible RT-PCR instruments" on page 10.

6. Open the experiment template file and save a separate copy with your experimental details. Run the experiment using Standard method for 384-well plates with the TaqMan® Gene Expression Master Mix and Fast mode for 96-well plates with the TaqMan® Fast Advanced™ Master Mix, using the cycling parameters listed below.

Note: The experiment template files (.eds) are available at **thermofisher.com/scorecardinstrument**.
Refer to the appropriate instrument user guide for information on how to set up the plate document/experiment or create a template from the setup file.

TaqMan® hPSC Scorecard™ 384w
Run mode (Ramp rate): Standard

Step	Temperature	Time	Cycles
Hold	50℃	2 minutes	—
Hold	95℃	10 minutes	—
Melt	95℃	15 seconds	40
Anneal/Extend	60℃	1 minute	

TaqMan® hPSC Scorecard™ 96w FAST
Run mode (Ramp rate): Fast

Step	Temperature	Time	Cycles
Hold	95℃	2 minutes	—
Melt	95℃	1 second	40
Anneal/Extend	60℃	20 seconds	

IMPORTANT! Be sure to run your qRT-PCR experiment using Standard Curve method. **Do not use ΔΔCt comparative PCR.**

Analyze the results

Analyze the gene expression data from the TaqMan® hPSC Scorecard™ Panels using the web-based hPSC Scorecard™ Analysis Software, available at **https://apps.thermofisher.com/hPSCscorecard/home.htm**.

The hPSC Scorecard™ Analysis Software summarizes all key experimental results, including pluripotency and differentiation potential on a single dashboard. It also allows you to tag and filter experiments, view expression, correlation, and box plots, and export experimental results and data as a PDF or as a spreadsheet.

For more information, refer to **thermofisher.com/us/en/home/life-science/stem-cell-research/taqman-hpsc-scorecard-panel/scorecard-software.html**. For guidance on how to interpret your results refer to the online user manual at **thermofisher.com/scorecardsoftwaremanual**.

Recipes

Media and reagents

Basic FGF stock solution

1. To prepare 10 mL of 10-µg/mL Basic FGF solution, aseptically mix the following components:

Basic FGF	100 µg
DPBS without Ca^{2+} and Mg^{2+}	9.8 mL
10% BSA	100 µL

2. Aliquot and store the Basic FGF solution at –20℃ for up to 6 months.

0.5 mM EDTA in DPBS

1. To prepare 50 mL of 0.5 mM EDTA in DPBS, aseptically mix the following components in a 50-mL conical tube:

DPBS without Ca^{2+} and Mg2+	50 mL
0.5 M EDTA	50 µL

2. Filter-sterilize the solution through a 0.22-µm filter and store at room temperature for up to 6 months.

Collagenase type IV solution

10X Collagenase Type IV solution (10 mg/mL, for 50 mL)

1. Add 50 mL of DMEM/F-12 to 500 mg of Collagenase Type IV to make a 10 mg/mL stock solution (10X).
2. Gently vortex to suspend, and filter sterilize the solution through a 0.22-µm filter. This solution can be stored at 2–8℃ for up to 2 weeks, or it can be aliquoted and stored frozen at –20℃ until use.

   1X Collagenase Type IV solution (1 mg/mL, for 50 mL)
3. To prepare a 1 mg/mL working solution of Collagenase Type IV, dilute the 10X stock solution 1:10 in DMEM/F-12.
4. The working solution can be used for 2 weeks if properly stored at 2–8℃ (store in aliquots to avoid repeated warming).

MEF medium

To prepare 100 mL of complete MEF medium, aseptically mix the components listed below. Complete MEF medium can be stored at 2–8℃ for up to 1 week.

Component	Volume
DMEM/F-12 (1X) with GlutaMAX™-I	89 mL
FBS, ESC-Qualified	10 mL
MEM™ Non-essential Amino Acids Solution (10 mM)	1 mL
ß-Mercaptoethanol (1000X)	100 µL

ESC medium

To prepare 100 mL of complete ESC medium, aseptically mix the components listed below. Complete ESC medium can be stored at 2–8℃ for up to 1 week.

Component	Volume
DMEM/F-12 (1X) with GlutaMAX™-I	79 mL
KnockOut™ Serum Replacement (KSR)	20 mL
MEM™ Non-essential Amino Acids Solution (10 mM)	1 mL
ß-Mercaptoethanol (1000X)	100 µL
Basic FGF[1] (10 µg/mL)	40 µL

[1] Prepare the iPSC Medium without bFGF, and then supplement with fresh bFGF to a final concentration of 4 ng/mL when the medium is used.

MEF-conditioned medium (MEF-CM)

1. Cover the whole surface of each new culture vessel with Attachment Factor (AF) solution and incubate the vessels for 30 minutes at 37℃ or for 1 hour at room temperature. For MEF-CM generation, a T-175 flask is recommended.

   Note: AF (Cat. no. S-006-100) is a sterile 1X solution containing 0.1 % gelatin (see “Media, sera, and supplements“ on page 42 for ordering information).

2. Using sterile technique in a laminar flow culture hood, completely remove the AF solution from the culture vessel by aspiration just prior to use. Coated vessels may be used immediately or stored at room temperature for up to 24 hours.

   Note: It is not necessary to wash the culture surface before adding cells or medium.

3. Plate 9.4 × 10^6 Mitomycin C-treated or irradiated MEFs in a T-175 flask coated with AF and containing 45 mL of MEF medium. Allow the cells to attach overnight in the incubator under normal growth conditions.

4. The following day, replace the MEF medium with 90 mL of ESC medium.

5. Collect the ESC medium, now considered MEF-CM, from the flasks after 24 hours of conditioning. This method of producing MEF-CM can be repeated up to seven days in a row.

6. Each day, filter-sterilize the collected MEF-CM through a 0.22 µM filter. Filtered MEF-CM can be used immediately or stored at –20℃ until use.

7. At the time of use, supplement the MEF-CM with fresh bFGF at a final concentration of 4 ng/mL.

StemPro™ hESC medium

To prepare 100 mL of complete StemPro™ hESC medium, aseptically mix the following components. StemPro™ hESC medium (without bFGF) can be stored at 2–8℃ for up to 2 weeks.

Component	Volume
DMEM/F-12 with HEPES	90.8 mL
StemPro™ hESC Supplement	2.0 mL
BSA 25%	7.2 mL
ß-Mercaptoethanol (55 mM)	182 µL
Basic FGF (10 µg/mL)[1]	80 µL

[1] Prepare the StemPro™ hESC medium without bFGF, and then supplement with fresh bFGF to a final concentration of 8 ng/mL when the medium is used.

StemPro™ wash solution

1. To prepare 100 mL of StemPro™ wash solution, aseptically mix the following components.

DMEM/F-12 with HEPES	100 mL
BSA 25%	0.2 mL

2. Filter-sterilize the solution through a 0.22-µm filter and store at 2–8℃ for up to 2 weeks.

Essential 8™ medium

1. Thaw the frozen Essential 8™ Supplement at 2–8℃ overnight. **Do not thaw the frozen supplement at 37℃.**

2. Mix the thawed supplement by gently inverting the vial a couple of times, remove 10 mL from the bottle of DMEM/F-12 (HAM) 1:1, and then aseptically transfer the entire contents of the Essential 8™ Supplement to the bottle of DMEM/F-12 (HAM) 1:1.

3. Swirl the bottle to mix and to obtain 500 mL of homogenous complete medium.

4. Complete Essential 8™ medium can be stored at 2–8℃ for up to 2 weeks. Before use, warm complete medium required for that day at room temperature until it is no longer cool to the touch. **Do not warm the medium at 37℃.**

Embryoid body (EB) medium

To prepare 100 mL of complete EB medium, aseptically mix the components listed below. Complete EB medium can be stored at 2–8°C for up to 1 week.

Component	Volume
DMEM/F-12 (1X) with GlutaMAX™-I	79 mL
KnockOut™ Serum Replacement (KSR)	20 mL
MEM™ Non-essential Amino Acids Solution (10 mM)	1 mL
ß-Mercaptoethanol (1000X)	100 µL

Preparing culture vessels

Coating culture vessels with Geltrex™ matrix

Coating protocol

1. Thaw a 5-mL bottle of Geltrex™ LDEV-Free hESC-Qualified Reduced Growth Factor Basement Membrane Matrix™ at 2–8℃ overnight.
2. Dilute the thawed Geltrex™ matrix solution 1:1 with cold sterile DMEM/F-12 to prepare 1-mL aliquots in tubes chilled on ice. These aliquots can be frozen at –20℃ or used immediately.

   Note: The aliquot volumes of the 1:1 diluted Geltrex™ matrix solution may be adjusted according to your needs.
3. To create working stocks, dilute an aliquot of Geltrex™ matrix solution 1:50 with cold DMEM on ice, for a total dilution of 1:100.

   Note: An optimal dilution of the Geltrex™ matrix solution may need to be determined for each cell line. Try various dilutions from 1:30 to 1:100.
4. Quickly cover the whole surface of each culture dish with the Geltrex™ matrix solution (see table).
5. Incubate the dishes in a 37℃, 5% CO_2 incubator for 1 hour.
6. Geltrex™ matrix-coated culture dishes can now be used or stored at 2–8℃ for up to a week. Do not allow dishes to dry.
7. Aspirate the diluted Geltrex™ matrix solution from the culture dish and discard. You do not need to rinse off the Geltrex™ matrix solution from the culture dish after removal. Cells can now be passaged directly onto the Geltrex™ matrix-coated culture dish.

Culture vessel	Surface area	Volume of Geltrex™ matrix dilution
6-well plate	10 cm^2/well	1.5 mL/well
12-well plate	4 cm^2/well	750 µL/well
24-well plate	2 cm^2/well	350 µL/well
35-mm dish	10 cm^2	1.5 mL
60-mm dish	20 cm^2	3.0 mL
100-mm dish	60 cm^2	6.0 mL

MEF culture dishes

Gelatin coating culture vessels

1. Cover the whole surface of each culture vessel with Attachment Factor (AF) solution and incubate the vessels for 30 minutes at 37°C or for 2 hours at room temperature.

   Note: AF (Cat. no. S-006-100) is a sterile 1X solution containing 0.1 % gelatin (see “Media, sera, and supplements“ on page 42 for ordering information).

2. Using sterile technique in a laminar flow culture hood, completely remove the AF solution from the culture vessel by aspiration.

   Note: It is not necessary to wash the culture surface before adding cells or medium. Coated vessels may be used immediately or wrapped in Parafilm™ sealing film and stored at room temperature for up to 24 hours.

Thawing MEFs

1. Remove the cryovial containing inactivated MEFs from the liquid nitrogen storage tank.
2. Briefly roll the vial between hands to remove frost, and swirl it gently in a 37°C water bath.
3. When only a small ice crystal remains in the vial, remove it from water bath. Spray the outside of the vial with 70% ethanol before placing it in the cell culture hood.
4. Pipet the thawed cells gently into a 15-mL conical tube.
5. Rinse the cryovial with 1 mL of pre-warmed MEF medium (see “MEF medium“ on page 34). Transfer the medium to the same 15-mL tube containing the cells.
6. Add 4 mL of pre-warmed MEF medium **dropwise** to the cells. Gently mix by pipetting up and down.

   Note: Adding the medium slowly helps the cells to avoid osmotic shock.

7. Centrifuge the cells at 200 × *g* for 5 minutes.
8. Aspirate the supernatant and resuspend the cell pellet in 5 mL of pre-warmed MEF medium.
9. Remove 20 µL of the cell suspension and determine the viable cell count using your method of choice (e.g., Countess™ Automated Cell Counter).

Plating MEFs

1. Centrifuge the remaining cell suspension (step 9 on page 38) at 200 × *g* for 5 minutes at room temperature.
2. Aspirate the supernatant. Resuspend the cell pellet in MEF medium (see “MEF medium“ on page 34) to a density of 2.5×10^6 cells/mL.
3. Aspirate the gelatin solution from the gelatin coated culture vessel.
4. Add the appropriate amount of MEF medium into each culture vessel (refer to the table).
5. Into each of these culture vessels, add the appropriate amount of MEF suspension (refer to the table).

   Note: The recommended plating density for Gibco™ Mouse Embryonic Fibroblasts (Irradiated) (Cat. no. S1520-100) is 2.5×10^4 cells/cm^2.
6. Move the culture vessels in several quick back-and-forth and side-to-side motions to disperse the cells across the surface of the vessels.
7. Incubate the cells in a 37°C incubator with a humidified atmosphere of 5% CO_2.
8. Use the MEF culture vessels within 3–4 days after plating.

Vessel size	Approximate growth area	Volume of MEF medium	Number of MEFs	Volume of MEF suspension
24-well plate	2 cm^2/well	0.5 mL	5.0×10^4/well	20 µL
12-well plate	4 cm^2/well	1 mL	1.0×10^5/well	40 µL
6-well plate	10 cm^2/well	2 mL	2.5×10^5/well	0.1 mL
60-mm dish	20 cm^2	5 mL	5.0×10^5	0.2 mL
100-mm dish	60 cm^2	10 mL	1.5×10^6	0.6 mL
25-cm^2 flask	25 cm^2	5 mL	6.3×10^5	0.25 mL
75-cm^2 flask	75 cm^2	15 mL	1.9×10^6	0.75 mL

Background information

TaqMan® chemistry

TaqMan® probes

TaqMan® probes are dual labeled, hydrolysis probes that increase the specificity of real-time PCR assays. TaqMan® probes contain:

- A reporter dye (for example, FAM™ dye) linked to the 5′ end of the probe
- A non-fluorescent quencher (NFQ) at the 3′ end of the probe
- MGB moiety attached to the NFQ

TaqMan® MGB probes also contain a minor groove binder (MGB) at the 3′ end of the probe. MGBs increase the melting temperature (Tm) without increasing probe length; allowing for the design of shorter probes.

How TaqMan® real-time chemistry works

1. An oligonucleotide probe is constructed with a fluorescent reporter dye bound to the 5′ end and a quencher on the 3′ end.

   While the probe is intact, the proximity of the quencher greatly reduces the fluorescence emitted by the reporter dye by fluorescence resonance energy transfer through space.

2. If the target sequence is present, the probe anneals between primer sites and is cleaved by the 5′ nuclease activity of the Taq DNA polymerase during extension. This cleavage of the probe:
   - Separates the reporter dye from the quencher, increasing the reporter dye signal.
   - Removes the probe from the target strand, allowing primer extension to continue to the end of the template strand. Thus, inclusion of the probe does not inhibit the overall PCR process.

3. Additional reporter dye molecules are cleaved from their respective probes with each cycle, resulting in an increase in fluorescence intensity proportional to the amount of amplicon produced. The higher the starting copy number of the nucleic acid target, the sooner a significant increase in fluorescence is observed.

Ordering information

Products

TaqMan® hPSC Scorecard™ panel products

Various components of the TaqMan® hPSC Scorecard™ Panels are also available separately. For more information about the following products, refer to our website at **thermofisher.com** or contact Technical Support.

Product	Quantity	Catalog no.
TaqMan® hPSC Scorecard™ Panel 384w	1 plate	A15870
TaqMan® hPSC Scorecard™ Kit 384w	1 kit	A15872
TaqMan® hPSC Scorecard™ Panel 2 × 96w FAST	2 plates	A15876
TaqMan® hPSC Scorecard™ Kit 2 × 96w FAST	1 kit	A15871
MicroAmp™ Optical Adhesive Film	25 covers 100 covers	4360954 4311971
TaqMan® Gene Expression Master Mix, 1-Pack (1 × 5 mL)	200 reactions	4369016
TaqMan® Fast Advanced™ Master Mix (1 × 1 mL)	100 reactions	4444556

Accessory products

For more information about the following products, refer to our website at **thermofisher.com** or contact Technical Support.

Product	Quantity	Catalog no.
High Capacity cDNA Reverse Transcription Kit with RNase Inhibitor	200 reactions 1000 reactions	4374966 4374967
TRIzol™ Reagent	100 mL 200 mL	15596-026 15596-018
DNA-*free*™ Kit	50 reactions	AM1906
UltraPure™ DNase/RNase-Free Distilled Water	500 mL 10 × 500 mL	10977-015 10977-023

Cells

For more information about the following products, refer to our website at **thermofisher.com** or contact Technical Support.

Product	Quantity	Catalog no.
Gibco™ Mouse Embryonic Fibroblasts (Irradiated) (1×10^6 cells/mL)	1 mL	S1520-100

Media, sera, and supplements

For more information about the following products, refer to our website at **thermofisher.com** or contact Technical Support.

Product	Quantity	Catalog no.
Dulbecco's Modified Eagle Medium (DMEM) with GlutaMAX™-I (high glucose)	500 mL	10569-010
DMEM/F-12 with GlutaMAX™-I	500 mL	10565-018
KnockOut™ Serum Replacement	100 mL 500 mL	10828-010 10828-028
Essential 8™ Medium (Prototype) (50X)	500 mL	A14666SA
StemPro™ hESC SFM™ Kit (for 500 mL of complete StemPro™ hESC SFM™)	1 kit	A1000701
MEM™ Non-Essential™ Amino Acids Solution (10 mM)	100 mL	11140-050
Basic Fibroblast Growth Factor (bFGF), recombinant human	10 µg	PHG0264
Bovine Albumin Fraction V Solution (BSA), 7.5%	100 mL	15260-037
Fetal Bovine Serum (FBS), ES-Cell Qualified	500 mL	16141-079
ß-Mercaptoethanol (1000X), liquid	50 mL	21985-023
DPBS, no Calcium, no Magnesium	500 mL	14190-144

Matrices and dissociation reagents

For more information about the following products, refer to our website at **thermofisher.com** or contact Technical Support.

Product	Quantity	Catalog no.
Geltrex™ hESC-qualified Reduced Growth Factor Basement Membrane Matrix™	5 mL	A1413302
Attachment Factor	100 mL	S-006-100
UltraPure™ 0.5 M EDTA, pH 8.0	4 × 100 mL	15575-020
0.05% Trypsin-EDTA (1X), Phenol Red	100 mL	25300-054
Collagenase, Type IV, powder	1 g	17104-019

Equipment

For more information about the following products, refer to our website at **thermofisher.com** or contact Technical Support.

Product	Quantity	Catalog no.
Countess™ Automated Cell Counter	1 unit	C10227
StemPro™ EZPassage™ Disposable Stem Cell Passaging Tool	10 units	23181-010

Safety

WARNING! GENERAL SAFETY. Using this product in a manner not specified in the user documentation may result in personal injury or damage to the instrument or device. Ensure that anyone using this product has received instructions in general safety practices for laboratories and the safety information provided in this document.

- Before using an instrument or device, read and understand the safety information provided in the user documentation provided by the manufacturer of the instrument or device.
- Before handling chemicals, read and understand all applicable Safety Data Sheets (SDSs) and use appropriate personal protective equipment (gloves, gowns, eye protection, and so on). To obtain SDSs, see the "Documentation and Support" section in this document.

Chemical safety

WARNING! GENERAL CHEMICAL HANDLING. To minimize hazards, ensure laboratory personnel read and practice the general safety guidelines for chemical usage, storage, and waste provided below. Consult the relevant SDS for specific precautions and instructions:

- Read and understand the Safety Data Sheets (SDSs) provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials. To obtain SDSs, see the “Documentation and Support” section in this document.
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing).
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with adequate ventilation (for example, fume hood).
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer's cleanup procedures as recommended in the SDS.
- Handle chemical wastes in a fume hood.
- Ensure use of primary and secondary waste containers. (A primary waste container holds the immediate waste. A secondary container contains spills or leaks from the primary container. Both containers must be compatible with the waste material and meet federal, state, and local requirements for container storage.)
- After emptying a waste container, seal it with the cap provided.
- Characterize (by analysis if necessary) the waste generated by the particular applications, reagents, and substrates used in your laboratory.
- Ensure that the waste is stored, transferred, transported, and disposed of according to all local, state/provincial, and/or national regulations.
- **IMPORTANT!** Radioactive or biohazardous materials may require special handling, and disposal limitations may apply.

Biological hazard safety

WARNING! BIOHAZARD. Biological samples such as tissues, body fluids, infectious agents, and blood of humans and other animals have the potential to transmit infectious diseases. Conduct all work in properly equipped facilities with the appropriate safety equipment (for example, physical containment devices). Safety equipment can also include items for personal protection, such as gloves, coats, gowns, shoe covers, boots, respirators, face shields, safety glasses, or goggles. Individuals should be trained according to applicable regulatory and company/ institution requirements before working with potentially biohazardous materials. Follow all applicable local, state/provincial, and/or national regulations. The following references provide general guidelines when handling biological samples in laboratory environment.

- U.S. Department of Health and Human Services, *Biosafety in Microbiological and Biomedical Laboratories (BMBL)*, 5th Edition, HHS Publication No. (CDC) 21-1112, Revised December 2009; found at:
  www.cdc.gov/biosafety/publications/bmbl5/BMBL.pdf
- World Health Organization, *Laboratory Biosafety Manual*, 3rd Edition, WHO/CDS/CSR/LYO/2004.11; found at:
  www.who.int/csr/resources/publications/biosafety/Biosafety7.pdf

Documentation and support

Customer and technical support

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- Product documentation
  - User guides, manuals, and protocols
  - Certificates of Analysis
  - Safety Data Sheets (SDSs; also known as MSDSs)

    Note: For SDSs for reagents and chemicals from other manufacturers, contact the manufacturer.

Limited product warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale at **www.thermofisher.com/us/en/home/global/terms-and-conditions.html**. If you have any questions, please contact Life Technologies at **www.thermofisher.com/support**.

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