Human Pluripotent Stem Cell Trilineage Differentiation qPCR Array

For characterization of human pluripotent stem cell trilineage differentiation to endoderm, mesoderm, and ectoderm germ layers

Catalog #07515 1 Kit

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Product Description

Human Pluripotent Stem Cell (hPSC) Trilineage Differentiation Quantitative Polymerase Chain Reaction (qPCR) Array is designed for characterization of hPSCs and their trilineage differentiation capacity. hPSCs, including human embryonic stem cells (hESCs) and human induced pluripotent stem cells (hiPSCs), are self-renewing and have the ability to differentiate into cells of the three embryonic germ layers: ectoderm, mesoderm, and endoderm. This qPCR array is designed for characterization of the gene expression profile of undifferentiated hESCs and hiPSCs and their trilineage derivatives following in vitro directed or spontaneous differentiation, thereby validating the ability of a cell line to differentiate to the three germ layers. Genes were selected based on their demonstrated differential expression in hESCs and hiPSCs compared with hiPSC-derived ectodermal, mesodermal, and endodermal lineage cells (Adewumi et al.; Bock et al.).

qPCR is a method for determining changes in steady-state mRNA levels of gene expression across multiple samples, generally normalized to the relative expression of internal control genes. Gene-specific primers are used in PCR to amplify target sequences within cDNA pools reverse-transcribed from mRNA. These PCR products contain hybridized sequence-specific probes that provide a fluorescent signal. Similar to TaqMan® technology, the fluorescent signal results from the 5' exonuclease activity of the Taq DNA polymerase on the probe, which is labeled with a reporter fluorophore (FAM) at the 5' end and a quencher (ZEN/IBFQ) at the 3' end. The rate of accumulation of the fluorescent signal is used to quantify the amount of cDNA present in the sample, and thereby the amount of mRNA present in the original cell lysate.

This 384-well qPCR array consists of 4 x 96-well block replicates. Each 96-well quadrant contains validated primers and probes for detection of 90 genes whose expression is correlated with undifferentiated hPSCs or their derivatives undergoing the early stages of differentiation. There are also 24 wells (6 per quadrant) containing primers and probes for endogenous (housekeeping) control genes. TATA box-binding protein (TBP) qPCR Array Control Template is provided separately as a synthetic DNA positive control, for use in a control well in each quadrant that contains primers and probes for TBP.

An annotated list of genes, as well as plate layouts and software for analysis of qPCR results, are available at www.stemcell.com/qPCRanalysis.

Product Information

The following components are sold as a complete kit (Catalog #07515) and are not available for individual sale.

NAME	COMPONENT #	SIZE	STORAGE	SHELF LIFE
TBP qPCR Control Template	07518	10^6 Copies	Store at -20°C.	Stable until expiry date (EXP) on label.
hPSC Trilineage Differentiation 384-Well qPCR Array Plate*	07502	1 Plate	Store at -20°C.	Stable until expiry date (EXP) on boxtop label.

*For instrument compatibility, visit www.stemcell.com/trilineageqPCRinstruments.

NOTE: Components may be shipped at room temperature (15 - 25°C) but should be stored at -20°C as indicated above.

Materials Required but Not Included

PRODUCT NAME	CATALOG #
Nuclease-Free Water (not DEPC-treated)	79001
Optical adhesive film	38108
qPCR Master Mix Kit • qPCR Master Mix (1 mL or 5 mL) • ROX Reference Dye (200 µL)	07516 (1 mL kit) OR 07517 (5 mL kit)
STEMscript™ cDNA Synthesis Kit with Oligo(dT) Primers OR STEMscript™ cDNA Synthesis Kit with Random Primers	79003 OR 79004
Total RNA Purification Kit	79040

Directions for Use

Please read the entire protocol before proceeding.

Use sterile technique when performing the following protocols.

Isolate RNA using the Total RNA Purification Kit. Quantify RNA by optical density at 260 nm, determine purity using $A_{260/280}$, then convert to cDNA using a STEMscript™ cDNA Synthesis Kit. Store cDNA at -20°C.

NOTE: We recommend synthesizing cDNA using an RNA concentration of 100 ng/µL in a 20 µL reverse transcription reaction. Use the synthesized cDNA as is in the qPCR reaction below or dilute up to 1 in 5 (Table 2).

NOTE: Each qPCR reaction requires 1 µL of cDNA template per well. For multiple wells, include 12.5% excess volume to account for pipetting loss (i.e. 108 µL total cDNA required for 96 wells). If the cDNA is diluted up to 1 in 5 prior to use, the required starting volume of undiluted cDNA is reduced proportionally (e.g. ~22 µL of undiluted cDNA is required to prepare 108 µL of diluted cDNA for 96 wells).

A. PREPARATION OF TBP qPCR CONTROL COCKTAIL AND cDNA COCKTAIL

1. Thaw qPCR Master Mix, cDNA, and ROX Reference Dye (if using) on ice.
2. If using ROX Reference Dye, add to qPCR Master Mix according to Table 1. For instruments not listed, refer to the manufacturer's instructions.

Table 1. Recommended Reference Dye Concentration Level for PCR Systems and Volume of ROX Reference Dye to Add to qPCR Master Mix

PCR SYSTEM	REFERENCE DYE CONCENTRATION LEVEL AND VOLUME OF ROX REFERENCE DYE (µL)/1 mL qPCR Master Mix		
	HIGH (40 µL)	LOW (4 µL)	NONE
Applied Biosystems • 7300 Real-Time PCR Systems	X		
Applied Biosystems • ViiA™ 7 and 7500 Real-Time PCR Systems • QuantStudio™ Systems		X	
Agilent Technologies • Mx3005P and Mx4000P		X	
Bio-Rad • CFX, iQ™, and DNA Engine Opticon® Real Time PCR Systems			X
Roche • LightCycler® Real-Time PCR System			X

3. Swirl bottle of qPCR Master Mix to mix thoroughly.
4. Prepare **TBP qPCR Control Cocktail** as follows:
   a. Centrifuge TBP qPCR Control Template at 3000 x g for 3 - 5 seconds to pellet material to the bottom of the vial.
   b. Add 20 µL of nuclease-free water and 20 µL of qPCR Master Mix to the vial. Vortex the vial gently and thoroughly to resuspend the pellet.
   c. Centrifuge at 3000 x g for 3 - 5 seconds to bring the liquid to the bottom of the vial.
5. Prepare **cDNA Cocktail** as follows:
   a. Mix cDNA by gently pipetting up and down. Centrifuge at 3000 x g for 3 - 5 seconds to bring liquid to the bottom of the vial.
   b. To a 15 mL conical tube (e.g. Catalog #38009), add components according to Table 2.

Table 2. Preparation of cDNA Cocktail

cDNA COCKTAIL COMPONENTS	VOLUME (µL)		
	1 well	96 wells	384 wells
cDNA	1	108	432
qPCR Master Mix	5	540	2160
Nuclease-free water	4	432	1728
Total Volume	**10**	**1080**	**4320**

   c. Cap the tube then gently vortex to mix thoroughly.
   Centrifuge at 3000 x g for 3 - 5 seconds to bring the liquid to the bottom of the tube.

B. PREPARATION OF qPCR PLATE

1. Carefully remove qPCR array plate from the box and plastic bag. Leave adhesive seal attached.
2. Centrifuge the plate at 1000 x g for 1 minute in a swinging bucket rotor fitted with plate holders. Ensure the plate is well balanced.
3. Carefully remove and discard the adhesive seal on the plate.
4. Using a multichannel pipettor (e.g. Catalog #38064) and reagent reservoir (e.g. Catalog #38080), dispense reagents (from section A) into the plate wells as described below.
   - 10 µL **TBP qPCR Control Cocktail** in each of wells H12, H24, P12, and P24 (see Figure 1)
   - 10 µL **cDNA Cocktail** in all other wells

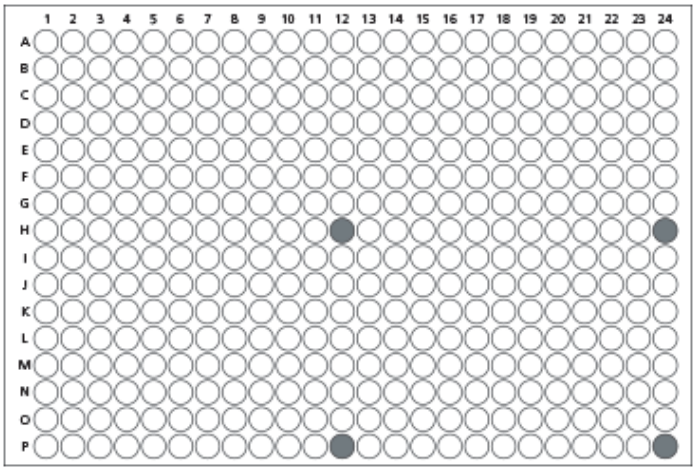

Figure 1. 384-Well Plate Diagram Indicating Wells Containing TBP qPCR Control Cocktail

5. Carefully cover and seal the plate using optical adhesive film.
6. Centrifuge the sealed plate at 1000 x g for 1 minute at room temperature (15 - 25°C) to remove bubbles from the bottom of the wells.
   NOTE: Bubbles in the bottom of the wells will interfere with results.
7. Place the plate on ice.

C. qPCR

1. Program the thermocycler as indicated in Table 3.

Table 3. Recommended qPCR Cycling Conditions

STEP	TEMPERATURE	TIME	
		FAST CYCLING	STANDARD CYCLING
Polymerase activation (1 cycle)	95°C	3 minutes	
Denaturation and annealing/extension (40 - 45 cycles)	95°C	5 seconds	15 seconds
	60°C*	30 seconds*	1 minute*
Hold	4°C	Up to 24 hours	

*Annealing/extension temperature or time may need to be adjusted based on primer sequences.

2. If ROX Reference Dye is being used, calibrate thermocycler.
3. Add plate and run PCR program.
4. Save file including Ct (cycle threshold) values.
5. Import the Ct data from the qPCR instrument to the analysis tool available at www.stemcell.com/qPCRanalysis. This analysis tool can rapidly and accurately quantitate relative gene expression, and the user can change analysis settings with ease.

Related Products

For related products, including specialized cell culture and storage media, matrices, antibodies, cytokines, and small molecules, visit www.stemcell.com/hPSCworkflow, or contact us at techsupport@stemcell.com.

References

Adewumi O et al. (2007) Characterization of human embryonic stem cell lines by the International Stem Cell Initiative. Nat Biotechnol 25(7): 803–16.

Bock C et al. (2011) Reference maps of human ES and iPS cell variation enable high-throughput characterization of pluripotent cell lines. Cell 144(3): 439–52.