Human Pluripotent Stem Cell-Derived Endoderm qPCR Array

For characterization of hPSC-derived progenitor cells of definitive endoderm, hepatic, intestinal, or pancreatic lineages

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Product Description

The Human Pluripotent Stem Cell (hPSC)-Derived Endoderm Quantitative Polymerase Chain Reaction (qPCR) Array is designed for characterization of hPSC-derived definitive endodermal progenitor cells and their differentiated progeny including pancreatic, hepatic, and intestinal cells. This qPCR array is designed for characterization of the gene expression profile of hPSC-derived endodermal cells following in vitro differentiation, and can aid in development of protocols for directed differentiation towards endodermal lineages. Genes were selected based on their demonstrated expression in hPSCs, or in hPSC-derived ectodermal, mesodermal, and endodermal lineage cells (D'Amour et al.), including pancreatic (Kroon et al.), hepatic (Hay et al.), and intestinal progenitor cells (Grapin-Botton & Melton).

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 fluorophore (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 qPCR array contains validated primers and probes for detection of 90 genes whose expression is correlated with hPSCs or hPSC-derived ectodermal, mesodermal, and endodermal cells, or pancreatic, hepatic, and intestinal progenitor cells. There are also 6 wells containing primers and probes for endogenous (housekeeping) control genes. TBP (TATA box-binding protein) qPCR Array Control Template is provided separately as a synthetic DNA positive control, for use in a control well containing 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.

Ordering Information

All kits listed below include TBP qPCR Control Template (Component #07518). For instrument compatibility, visit www.stemcell.com/DEqPCRinstruments.

KIT CATALOG #	PLATE COMPONENT #	SIZE
07531	07505.1	1 Plate (96 wells)
07533	07505.3	1 Plate (96 wells)
07534	07505.4	1 Plate (96 wells)

Storage and Stability

Store plates at -20°C. Stable until expiry date (EXP) on boxtop label.

Store TBP qPCR Control Template at -20°C. Stable until expiry date (EXP) on label.

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 #
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
Nuclease-Free Water (not DEPC-treated)	79001
Optical adhesive film	38108

Directions for Use

Please read the entire protocol before proceeding.

Use sterile techniques when performing the following protocols.

Isolate RNA using standard protocols. 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: Optimal concentration of cDNA for qPCR amplification is 20 - 100 ng/µL.

A. PREPARATION OF TBP qPCR CONTROL TEMPLATE 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. Volume of ROX Reference Dye to add to qPCR Master Mix

PCR SYSTEM	VOLUME OF ROX REFERENCE DYE (µL)	
	1 mL qPCR Master Mix	5 mL qPCR Master Mix
7900HT Fast (Applied Biosystems)	40	200
ViiA™ 7 (Applied Biosystems)	4	20
QuantStudio™ Flex (Applied Biosystems)	4	20

3. Swirl bottle of qPCR Master Mix to mix thoroughly.
4. Prepare **TBP qPCR Control Template** 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 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*
cDNA	1	108
qPCR Master Mix	5	540
Nuclease-free water	4	432
Total Volume	**10**	**1080**

*12.5% excess volume added to account for pipetting dead volume

c. Cap the tube then gently vortex to mix thoroughly.
d. 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.
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.
   - 5 µL TBP qPCR Control Template + 5 µL qPCR Master Mix in well H12 (see Figure 1)
   - 10 µL cDNA Cocktail in all other wells

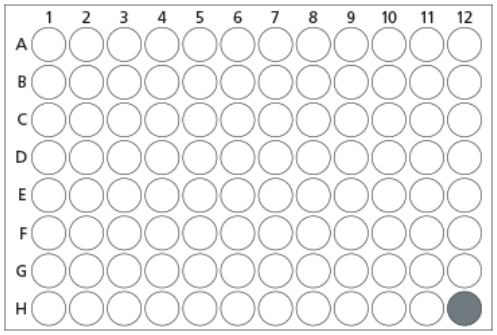

Figure 1. 96-Well Plate Diagram Indicating Well Containing TBP qPCR Control Template

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 (for Catalog #07531) or Table 4 (for Catalog #07533 or 07534).

Table 1. Recommended qPCR Cycling Conditions (for Catalog #07531)

STEP	TEMPERATURE	TIME
Polymerase activation (1 cycle)	95°C	20 seconds
Denaturation and annealing/extension (40 - 50 cycles)	95°C	1 second
	60°C*	20 seconds*
Hold	4°C	Up to 24 hours

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

Table 2. Recommended qPCR Cycling Conditions (for Catalog #07533 or 07534)

STEP	TEMPERATURE	TIME	
		Fast Cycling	Standard Cycling
Polymerase activation (1 cycle)	95°C	3 minutes	
Denaturation and annealing/extension (35 - 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 media, matrices, antibodies, cytokines, and small molecules, visit www.stemcell.com/DEworkflow or contact us at techsupport@stemcell.com.

References

D'Amour KA et al. (2005) Efficient differentiation of human embryonic stem cells to definitive endoderm. Nat Biotechnol 23(12): 1534–41.

Grapin-Botton A & Melton DA. (2000) Endoderm development: from patterning to organogenesis. Trends Genet 16(3): 124–30.

Hay DC et al. (2008) Highly efficient differentiation of hESCs to functional hepatic endoderm requires ActivinA and Wnt3a signaling. Proc Natl Acad Sci USA 105(34): 12301–6.

Kroon E et al. (2008) Pancreatic endoderm derived from human embryonic stem cells generates glucose-responsive insulin-secreting cells in vivo. Nat Biotechnol 26(4): 443–52.