

TaqMan® hPSC Scorecard™ Panel

Cat. nos. A15870, A15871, A15872, A15876

Doc. Part no. 100019866 **Pub. no.** MAN0008318

Rev. 1.0

Detailed protocol is available at lifetechnologies.com/manuals.

For additional product information and resources, visit lifetechnogies.com/scorecard.

For research use only. Not for use in diagnostic procedures.

TagMan® hPSC Scorecard™ Panel Workflow

Detailed protocol is available at www.lifetechnologies.com/manuals.

ESC/iPSC Sample - RNA Isolation - DNase Treatment - cDNA Preparation

Undifferentiated ESC/ iPSC

Differentiated ESC/

- Embryoid bodies (week 1) or
- Directly differentiated cells

Sample requirements:

>5 × 10⁵ cells/sample

TRIzol® Organic Phase Extraction

- Harvest sample with TRIzol® reagent (Cat. no. 15596).
- Extract RNA using chloroform.
- 3. Precipitate RNA in 100% isopropanol
- 4. Wash RNA in 75% ethanol.
- 5. Resuspend RNA in 20-50 µL RNase-free water

Sample requirements:

1 mL TRIzol® reagent per 6-cm culture dish

DNA-free[™] Kit (optional) (Cat. no. AM1906)

- Remove contaminating DNA from the RNA sample.
- Measure A₂₆₀ and A₂₈₀ to determine total RNA amount and quality.

[RNA] (µg/mL):

 $A_{260} \times 40 \times \text{dilution factor}$

Recommended ratio: $A_{260}/A_{280} = -2.0$

TaqMan® qRT-PCR

hPSC Scorecard™ Panel Experiment Type:

Standard Curve

qPCR Setup:

- Add 20 µL water and 70 µL 2× master mix per RT reaction (i.e., cDNA sample; total final V = 140 µL).
- water and 225 µL 2× RT master mix.

 2. Aliquot 50 µL RT reaction mix per well or PCR tube.

High Capacity cDNA RT

Kit with RNase Inhibitor

8 reverse transcription

(RT) reactions per RNA

1. Dilute 1 ua RNA in

225 uL RNase-free

(Cat. no. 4374966)

sample:

3. Perform RT reaction using the following conditions:

25°C - 10 min 37°C - 120 min 85°C - 5 min 4°C - hold

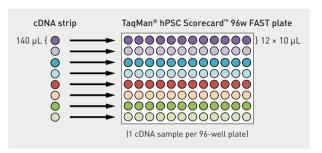
- total final V = 140 µL).

 96-wells: TaqMan®
 Fast Advanced
 Master Mix
- 384-wells: TaqMan® Gene Expression Master Mix (Cat. no. 4369016)

(Cat. no. 4444557)

- 2. Load 10 µL per well into hPSC Scorecard™ plate (see loading quide below).
- Run plate (see cycling parameters below).

TaqMan® hPSC Scorecard™ Panel loading guide



Cycling parameters

TaqMan® hPSC Scorecard™ 96w FAST [Cat. nos. A15876, A15871]

Run Mode: Fast

TaqMan® Fast Advanced Master Mix (Cat. no. 4444557)

(001.110.4444007)				
	PCR (40 cycles)			
Hold	Melt	Anneal/extend		
95°C	95°C	60°C		
20 sec	1 sec	20 sec		

(4 cDNA samples per 384-well plate)

TaqMan® hPSC Scorecard™ 384w (Cat. nos. A15870, A15872)

Run Mode: Standard

TaqMan® Gene Expression Master Mix

		PCR (40 cycles)	
Hold	Hold	Melt	Anneal/extend
50°C	95°C	95°C	60°C
2 min	10 min	15 sec	1 min

WARNING! For every chemical, read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from www.lifetechnologies.com/support.

Documentation and Support

For additional product and technical information, such as Safety Data Sheets (SDS), Certificates of Analysis, etc., visit our website at www.lifetechnologies.com. For further assistance, email our Technical Support team at techsupport@lifetech.com.

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