

TaqMan® Protein Expression Assays

Key Features Include:

Sensitivity—requires 10–100 times less sample than alternative methods

Simplicity—one-step lysis sample preparation and streamlined real-time PCR workflow enables sample-to-data in 3.5 hours

Quantitative Results—TaqMan® 5' nuclease chemistry is the gold standard for quantitative results

Flexibility—expands beyond RNA to protein using the same instrument platform



Figure 1. The new TaqMan® Protein Expression Assays family helps provide fast and easy identification and relative quantification of protein markers from limited quantities of cultured human embryonic stem cells.

Introduction

Human embryonic stem cell (hESC) characterization is an emerging field that, until now, required researchers to use labor-intensive protein analysis techniques such as flow cytometry, immunostaining, and western blot analysis. These methods lack sensitivity and are not inherently quantitative. TaqMan® Protein Expression Assays enable fast and easy identification and relative protein quantification of pluripotency markers from limited quantities of cultured human embryonic stem cells (hESCs). These assays enable an integrated qPCR approach for measuring relative changes in gene and protein expression from the same starting sample and on a single analytical platform that can also run TaqMan® Gene Expression Assays.

Gold Standard TaqMan® Assays for Stem Cell Research

By making novel adaptations in assay design, Applied Biosystems brings the gold standard specificity, sensitivity, and reproducibility of TaqMan® Assays and quantitative real-time PCR to stem cell protein research. Revolutionary TaqMan® Protein Expression Assays help quantitate protein expression using TaqMan® 5' nuclease chemistry with a workflow and sample quantity similar to our mRNA and microRNA (miRNA) assays. As the protein expression results are obtained on the same analytical platform, these new assays enable direct correlation of mRNA and/or miRNA expression to protein expression. No other quantitative protein analysis method can offer this capability.

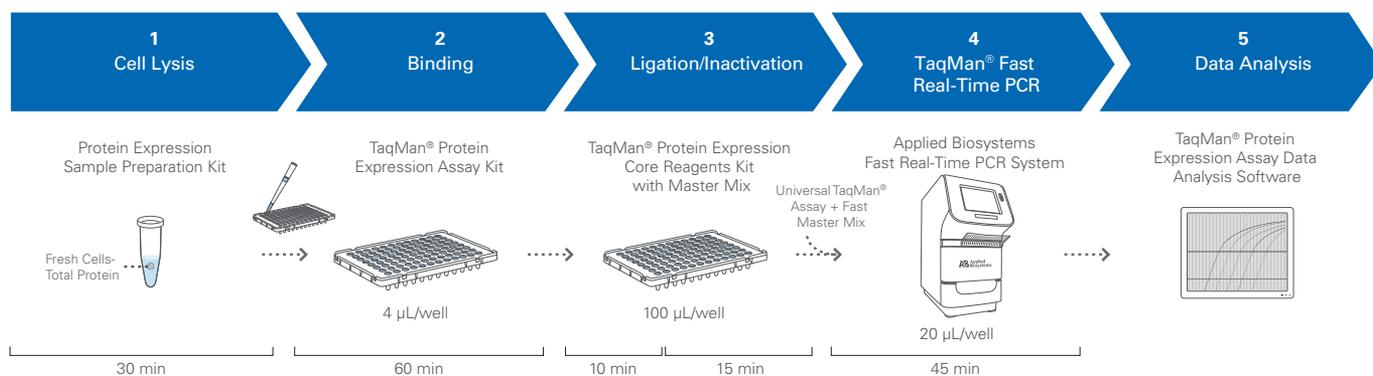


Figure 2. Detailed Workflow of the TaqMan[®] Protein Expression Assay Protocol. Only 2 µL of starting sample (10–500 cells/µL) is required for each reaction well. For the TaqMan[®] Assay in step 4, 10 µL of the ligated sample is combined with the master mix for a final real-time PCR reaction of 20 µL.

Furthermore, these pre-designed assays are straightforward and robust, with no washing steps, and require 100–500 times fewer cells than typically required with western blot approaches. As with traditional gene expression TaqMan[®] Assays, the data obtained from TaqMan[®] Protein Expression Assays are readily amenable to relative quantification, and are ideally suited for the relative quantification of markers in pluripotent and differentiated stem cells.

Streamlined Workflow

The TaqMan[®] Protein Expression Assay workflow consists of a simple, one-step sample lysis protocol that releases all classes of proteins, which is combined with TaqMan[®] chemistry to create a highly sensitive and specific process for measuring protein expression in small samples. The entire experiment process, from sample to data, can be completed in about 3.5 hours (Figure 2), with a typical hands-on time of only 1.5 hours.* All reagents have been optimized for assaying proteins from

cultured cells. The sample preparation is a gentle, one-step cell lysis with nonionic detergent. The samples are then ready to be directly mixed with the assay probes from any Protein Expression Assay Kit, where binding of the assay probes takes 60 minutes. Then, ligase is added to link the oligonucleotides together, followed by a 15-minute inactivation or clean-up step, which digests all the proteins. Next, a single plate transfer is performed into the PCR plate where Fast real-time PCR is performed.

**Results may vary depending on the circumstances of the user.*

Convenient, Easy-to-Use Assays

Pre-designed TaqMan[®] Protein Expression Assays have been developed for several human protein targets, including hICAM1, hCSTB, hOCT3/4, hNANOG, hSOX2, and hLIN28, along with supporting chemistry reagents to monitor the relative protein levels in human embryonal stem cells. The TaqMan[®] Protein Expression Assay product line also includes a core reagents kit, positive and negative control sample cell lysate kits, and free data analysis software (Table 1).

Table 1. The Main Components of the TaqMan[®] Protein Expression Assay Product Line.

TaqMan [®] Protein Expression Assays pre-designed to human targets	
• human ICAM1	• human OCT3/4
• human CSTB	• human SOX2
• human NANOG	• human LIN28
Protein Expression Assay Core Reagents Kit	
• Required to run an assay along with each Protein Expression Assay Kit	
• Contains all the reagents needed to run the assays, including a connector oligo, ligase, a universal TaqMan [®] Assay, and a new TaqMan [®] Protein Expression Fast Master Mix	
Protein Expression Sample Preparation Kit	
• One-step lysis of cultured cells	
Positive and Negative Control Sample Cell Lysates	
• Protein Expression Lysate Control Kit (Raji)	
– positive sample lysate for CSTB, and ICAM1	
– negative sample lysate for OCT3/4, NANOG, SOX2, and LIN28	
• Protein Expression Lysate Control Kit (NTERA2)	
– positive sample lysate for OCT3/4, NANOG, SOX2, LIN28, CSTB, and ICAM1	
Data Analysis Software	
• TaqMan [®] Protein Expression Assays Data Analysis Software	

Reliable Real-Time PCR Platforms

TaqMan® Protein Expression Assays can be run on any Applied Biosystems Fast Real-Time PCR System to provide real-time PCR results in under one hour. Applied Biosystems Real-Time PCR Systems make real-time PCR more accessible than ever before by providing powerful solutions to fit the needs of any laboratory. These systems are easy to use with next generation software, and of course, they're backed by Applied Biosystems' unmatched track record of performance, quality, and long-term reliability. Complete product information on all our Fast Real-Time PCR Systems can be found at www.appliedbiosystems.com.

Intuitive Data Analysis Software

The sophisticated software package provided with all Applied Biosystems Real-Time PCR Systems facilitates experimental setup, data collection, and assay performance analysis. The free TaqMan® Protein Expression Assays Data Analysis Software tool can be downloaded at www.appliedbiosystems.com/taqman4protein.

Global Service and Support

Our products are backed by one of the world's most reliable service and support organizations. Our global service professionals are trained and dedicated to help you 24 hours a day, seven days a week—over the phone or on the Web.

For more information on TaqMan® Protein Expression Assays, visit us at www.appliedbiosystems.com/proteinassays.

Proven TaqMan® Chemistry

TaqMan® Assays feature 5' nuclease chemistry and TaqMan® probes that incorporate minor groove binder (MGB) technology and a non-fluorescent quencher (NFQ). The MGB probe enhances the probe melting temperature (T_m) by binding in the minor groove of a DNA duplex. This T_m enhancement enables the use of probes as short as 13 bases. Shorter probes offer superior probe/primer design for improved specificity. They also provide greater flexibility when designing assays for closely related sequences such as gene family members, or species-specific assays. The incorporation of an NFQ virtually eliminates the background fluorescence associated with traditional quenchers, providing better sensitivity and quantitation precision (Figure 3).

Tested for Optimum Performance

All TaqMan® Assays undergo rigorous quality-control testing. Mass spectrometry verifies the sequence, and further testing ensures proper formulation of probe and primer mix. The FAM™ dye-labeled TaqMan® MGB probe and two unlabeled PCR primers are then formulated into a ready-to-use, single-tube assay.

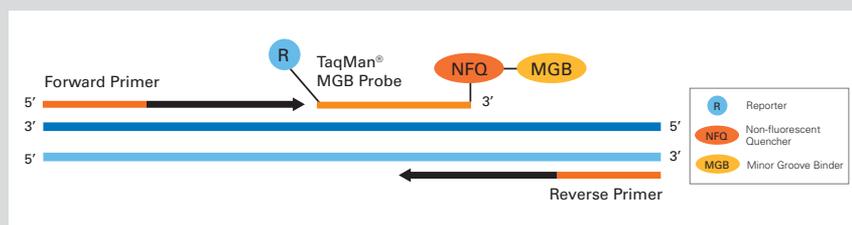


Figure 3. Schematic of a TaqMan® Assay.

ORDERING INFORMATION

Description	Size	P/N
Assay Kits		
TaqMan® Protein Expression Assay Kit (hCSTB)	100 rxns	4405465
TaqMan® Protein Expression Assay Kit (hICAM1)	100 rxns	4405471
TaqMan® Protein Expression Assay Kit (hOCT3/4)	100 rxns	4405489
TaqMan® Protein Expression Assay Kit (hNANOG)	100 rxns	4405483
TaqMan® Protein Expression Assay Kit (hSOX2)	100 rxns	4405495
TaqMan® Protein Expression Assay Kit (hLIN28)	100 rxns	4405477
Required for Use With Assay Kits		
TaqMan® Protein Expression Core Reagents Kit with Master Mix	100 rxns	4405501
TaqMan® Protein Expression Core Reagents Kit with Master Mix	5 pack, 500 rxns	4440475
Supporting Kits		
Protein Expression Sample Preparation Kit	1,000 rxns	4405443
Protein Expression Lysate Control Kit (Raji)	100 rxns	4405448
Protein Expression Lysate Control Kit (NTERA2)	100 rxns	4405454
Combination Starter Packs		
Protein Expression Starter Pack with Lysate Controls (contains two Protein Expression Lysate Control Kits (Raji and NTERA2) plus five complete assays—CSTB, OCT3/4, NANOG, SOX2, and LIN28)	500 assay rxns 200 lysate rxns	4440476
Protein Expression Starter Pack with Sample Preparation Kit (contains one Protein Expression Sample Preparation Kit plus five complete assays—CSTB, OCT3/4, NANOG, SOX2, and LIN28)	500 assay rxns 1,000 sample preparation rxns	4440477

For Research Use Only. Not for use in diagnostic procedures.

Practice of the patented 5' Nuclease Process requires a license from Applied Biosystems. The purchase of TaqMan® Protein Expression Assays includes an immunity from suit under patents specified in the product insert to use only the amount purchased for the purchaser's own internal research when used with the separate purchase of an Authorized 5' Nuclease Core Kit. No other patent rights are conveyed expressly, by implication, or by estoppel. For further information on purchasing licenses contact the Director of Licensing, Applied Biosystems, 850 Lincoln Centre Drive, Foster City, California 94404, USA.

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Headquarters

850 Lincoln Centre Drive | Foster City, CA 94404 USA
Phone 650.638.5800 | Toll Free 800.345.5224
www.appliedbiosystems.com

International Sales

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