

A revolutionary TaqMan® Assay product line that unlocks the power of real-time PCR for protein analysis: small sample protein quantitation using antibodies.

TaqMan® Protein Assays use antibodies and proximity ligation, expanding the range of quantitative PCR (qPCR) applications to include direct detection of proteins. TaqMan® Protein Assay chemistry helps bring the benefits and advantages of real-time PCR to analyzing very small samples with high sensitivity.

Used with Applied Biosystems® Real-Time PCR Systems, TaqMan® Protein Assays enable quick and easy identification and relative quantification of proteins from limited quantities of cell and tissue lysates. Additionally, the TaqMan® Protein Assays Open Kit enables you to make your own TaqMan® Protein Assay using your antibodies. These assays have successfully been used for a range of small sample applications—from monitoring cellular differentiation (loss of pluripotency), to monitoring gene silencing,

Table 1. Examples of protein assay sensitivity in buffer with proximity probes made utilizing commercially available antibodies and the TaqMan® Protein Assays Open Kit.

Target	Antibody Type	Vendor-Published LOD*	LOD	LOD/Assay
TNFRI	Pair	50 pg/mL	16 pg/mL	32 fg/well
DCN	Pair	30-50 pg/mL	13 pg/mL	26 fg/well
Human CSTD	Polyclonal	0.3 ng/well**	38 pg/mL	76 fg/well
Mouse CSTD	Polyclonal	NA	1.6 ng/mL	3.2 pg/well
SCF	Polyclonal	0.3 ng/well**	27 pg/mL	54 fg/well
CD117	Polyclonal	NA	14 pg/mL	28 fg/well
p53	Polyclonal	NA	130 pg/mL	0.26 pg/well
Pro-CASP3	Polyclonal	NA	400 pg/mL	0.8 pg/well
CASP8	Polyclonal	NA	1.4 ng/mL	2.8 pg/well
CDH1/E-cadherin	Polyclonal	1.0 ng/well**	4.0 pg/mL	8 fg/well

*Typical limit of detection (LOD) reported by the antibody vendor: calculated as 3 SD above background (NPC) on recombinant protein standard curves.

**Typical LOD reported by the antibody vendor: calculated as 3 SD above background (NPC) utilizing direct ELISA method.

transfection, and transduction, to screening for protein biomarkers and protein-protein interactions. Whether doing miRNA target analysis or screening cells for low-expression proteins, TaqMan® Protein Assay products provide a novel technology, enabling the highest sensitivity for quantitative protein analysis.

Features and Benefits

Feature	Benefit
Assays require only 2 µL starting input and measure protein expression in 1–500 cells (0.1 ng–50 ng total protein)	<ul style="list-style-type: none"> Only need a few thousand cells for a complete experiment Analyze several different protein targets with the same small amount of cells or tissue lysate
Open Kit “tool kit” provides the flexibility to make an assay to almost any target of interest	<ul style="list-style-type: none"> Make assays to targets of choice—specific to research needs Make assay probes in 90 minutes with biotinylated antibodies
Streamlined, homogeneous assay development	<ul style="list-style-type: none"> Make homogeneous assays from a single affinity-purified polyclonal antibody or a matched monoclonal antibody pair No washing steps required Make assay probes and run an assay in less than 1 day
A single TaqMan® PCR assay and optimized conditions provide a universal protocol for all assays	<ul style="list-style-type: none"> Assays are robust, reproducible, and sensitive Several assays can be run on the same plate Assays work under universal cycling conditions
Easily correlate mRNA and miRNA to protein with the same sample on the same qPCR platform	<ul style="list-style-type: none"> Protein assays follow a protocol similar to TaqMan® Gene Expression Assays Protein assay readout is on the same platform as mRNA and miRNA assays
Pre-designed assays are ready to go and characterized with positive and negative control cell lysate samples	<ul style="list-style-type: none"> No design expertise required Optimized assay performance Training and learning curve for new assays is easier

Technology Background

TaqMan® Protein Assays offer a novel way to detect proteins through the amplification of a surrogate DNA template. The assays utilize proximity ligation assay (PLA) technology for qPCR applications in the RUO field, and they transform target proteins present in a crude cell or tissue lysate into nucleic acid sequences conveniently detectable by DNA amplification. The TaqMan® Protein Assay probes for each individual assay consist of a pair of protein (analyte)-specific antibodies linked to reporter oligonucleotides, and they specifically bind the target protein on two different epitopes (antigen/

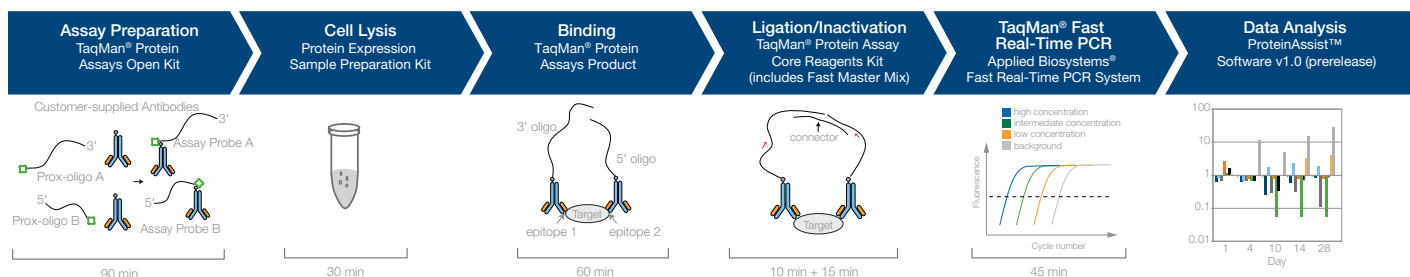


Figure 1. Proximity Ligation Assay Technology Overview. After making assay probes using customer-supplied biotinylated antibodies and the TaqMan® Protein Assays Open Kit, the sample is prepared with a simple one-step lysis of cells, and no further purification is required. The four primary steps of running the assay are: (1) binding of paired antibody-oligonucleotide probes to a protein target in cell or tissue lysates; (2) templated ligation of the oligonucleotides in proximity using a connector oligo and DNA ligase; (3) qPCR amplification and detection; (4) data analysis.

antibody recognition sites). The target-bound assay probes are united by enzymatic DNA ligation in a proximity-dependent reaction, forming DNA templates which are then amplified and analyzed by qPCR.

To utilize the TaqMan® Protein Assays Open Kit, you supply your own biotinylated antibodies and add them to streptavidin-oligonucleotides (prox-oligos) to make the two antibody-oligonucleotide assay probes utilized in binding.

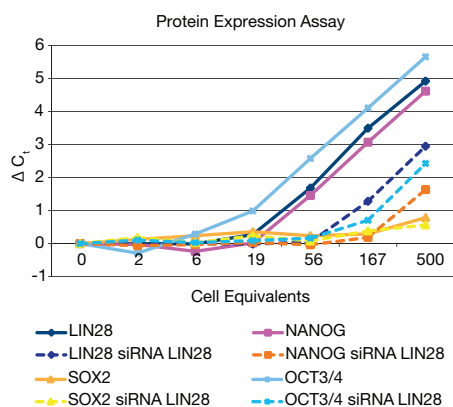


Figure 2. Demonstrated Pre-designed TaqMan® Protein Assay Sensitivity. TaqMan® Protein Assays were performed on four common biomarkers of the pluripotent state: human LIN28, NANOG, SOX2, and OCT3/4 proteins in TCam2 cells [a human seminoma cell line that is typically negative for expression of SOX2]. A lysate containing only 4,000 cells was required for all the assays [2 µL lysate/assay, at 250 cells/µL]. The four biomarker proteins were present at various concentrations, and the effect on these pluripotent markers was examined after transfecting the TCam2 cells with a Lin28 siRNA, which resulted in decreased expression of all of the proteins. The TaqMan® Protein Assays were highly reproducible (variation <0.2 C_t between technical replicates). Data courtesy of Dr. Leendert Looijenga, Erasmus University, The Netherlands.

ORDERING INFORMATION

Description	SIZE	CAT. NO.
TaqMan® Protein Assays Open Kit		
TaqMan® Protein Assays Open Kit	4000 rxns	4453745
TaqMan® Protein Assays Oligo Probe Kit	4000 rxns	4448549
TaqMan® Protein Assays Buffer Kit	4000 rxns	4448571
Supporting Kits		
Sample Prep Kits		
Protein Quant Sample Lysis Kit	25 mL	4448536
Protein Expression Sample Prep Kit	1000 rxns	4405443
Core Reagents Kits (required for running assays*)		
TaqMan® Protein Assays Core Reagents Kit w/ Master Mix	500 rxns	4448591
TaqMan® Protein Assays Core Reagents Base Kit	500 rxns	4448592
TaqMan® Protein Assays Fast Master Mix	500 rxns	4448616
TaqMan® Protein Assays Core Reagents Kit w/ Master Mix	100 rxns	4405501

TaqMan® Protein Assay technology is comparable to ELISAs in terms of sensitivity; however, the homogeneous format of TaqMan® Assays enables an easier workflow with no washing steps (Figure 1). In addition, the ability to make an assay from only a single polyclonal antibody (rather than a pair of antibodies) enables easier assay development. TaqMan® Protein Assays can also be made with ELISA pairs and, when tested with recombinant protein in buffer, result in equivalent or better sensitivity (Figure 2) and dynamic range compared to the standard ELISA format, while using less sample. Table 1 details assay sensitivity in buffer with assays made from commercially available antibodies and the TaqMan® Protein Assays Open Kit.

Ordering

TaqMan® Protein Assays and other related products can be found online at www.appliedbiosystems.com/taqman4antibodies.

Additional Product Information

www.appliedbiosystems.com/proteinassays

Technical Support: 800-327-3002, prompt 5

Related Literature

Brochure: TaqMan® Protein Assays	(PN C031111)
Chemistry Guide: Real-Time PCR Protein Expression Assays	(PN 4405780)
Protocol: TaqMan® Protein Assays Probe Development	(PN 4449282)
Protocol: TaqMan® Protein Assays and Sample Prep	(PN 4449283)
Quick Reference Card: TaqMan® Protein Assays Probe Development	(PN 4449772)
Quick Reference Card: TaqMan® Protein Assays	(PN 4449281)
Quick Reference Card: TaqMan® Protein Assays Sample Prep	(PN 4449771)

Reference

Swartzman E, Shannon M, Lieu P et al. [2010] Expanding applications of protein analysis using proximity ligation and qPCR. *Methods* 50(4):S23–6.

Description	SIZE	CAT. NO.
Core Reagents Kits (required for running assays*)		
TaqMan® Protein Assays Core Reagents Base Kit	100 rxns	4405460
TaqMan® Protein Assays Fast Master Mix	100 rxns	4400088
Control Lysate Kits		
Protein Expression Lysate Control Kit (Raji)	100 rxns	4405448
Protein Expression Lysate Control Kit (NTERA2)	100 rxns	4405454
TaqMan® Protein Assays (ready-to-use assays for proteins involved in stem cell analysis)		
TaqMan® Protein Assay Kit (hCSTB)	100 rxns	4405465
TaqMan® Protein Assay Kit (hICAM1)	100 rxns	4405471
TaqMan® Protein Assay Kit (hOCT3/4)	100 rxns	4405489
TaqMan® Protein Assay Kit (hNANOG)	100 rxns	4405483
TaqMan® Protein Assay Kit (hSOX2)	100 rxns	4405495
TaqMan® Protein Assay Kit (hLIN28)	100 rxns	4405477

*A TaqMan® Protein Core Reagents Kit with Master Mix is necessary for use with TaqMan® Protein Assay Kits.

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Practice of the patented 5' Nuclease Process requires a license from Applied Biosystems. The purchase of TaqMan® Protein 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. Products are covered by IP rights held by and licensed from Olink AB, Sweden.

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