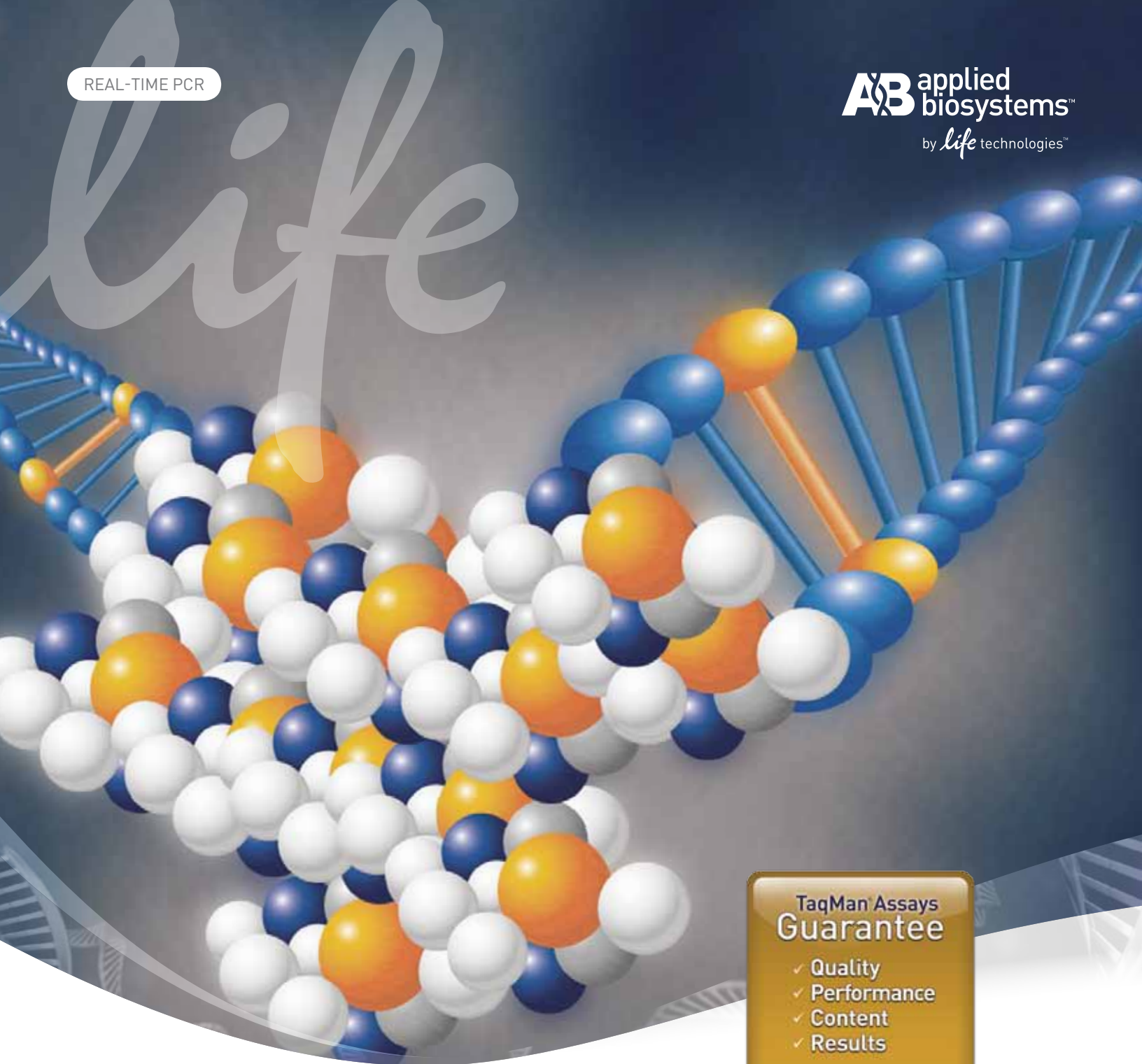


REAL-TIME PCR

AB applied
biosystems™
by *life* technologies™



TaqMan Assays
Guarantee

- ✓ Quality
- ✓ Performance
- ✓ Content
- ✓ Results

TaqMan® Protein Assays

Unlock the power of real-time PCR for protein analysis

life
technologies™

I can use my real-time PCR instrument to quantitate protein?

Do protein levels correlate with related mRNA levels in my experiments?

My samples are very small—how can I quantitate my proteins?



Is there an easy way to make a homogeneous assay from a single polyclonal antibody or a matched antibody pair?



I know my microRNA is expressed. How can I measure levels of potential target mRNAs and proteins?

Can I measure protein-protein interactions in solution?

Introducing TaqMan® Protein Assays: small-sample protein quantitation using real-time PCR and your antibodies

Targeted protein quantitation using a small amount of sample

Get specific, quantitative information about where and when proteins are expressed using limited samples

Correlation of RNA and protein levels

Follow up mRNA and siRNA experiments at the protein level

MicroRNA functional analysis

Evaluate the effects of microRNA (or other noncoding RNA) levels on potential target mRNA and protein quantities

Streamlined, homogeneous assay development

Make homogeneous protein assays from a single affinity-purified polyclonal antibody or a matched antibody pair

Direct protein–protein interaction screening

Screen *in vitro* protein–protein interactions directly using a TaqMan® Protein Assay made from antibodies for the two interacting proteins

Real-time PCR for protein

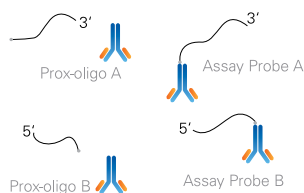
Changes in mRNA levels have long been monitored as early indicators in biological systems. Recently, microRNAs and other noncoding RNAs (both large and small) have emerged as important early modulators of many cellular responses as well. All of these RNA species can have an effect on the levels of their associated proteins.

The correlation of RNA and protein levels, however, has been challenged by the need for separate sample

handling and analysis platforms for nucleic acids and proteins. TaqMan® Protein Assays expand qPCR applications from familiar nucleic acid analysis to include protein detection and quantification.

The qPCR workflow is simple, rapid, and flexible compared to protein analysis methods such as immunostaining and western blotting. Another big advantage of TaqMan® Protein Assays over these technologies is that the data are quantitative and easier to interpret.

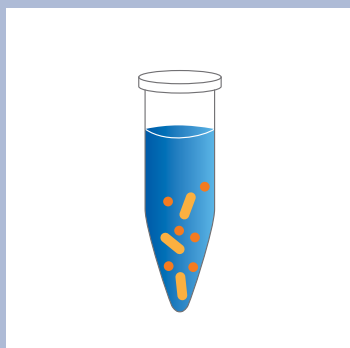
Customer-supplied Antibodies



Build your assay 90 min*

Biotinylated antibodies specific for the protein of interest are attached to oligonucleotides using a biotin-streptavidin interaction.

- **TaqMan® Protein Assays Open Kit**



Prepare sample 30 min*

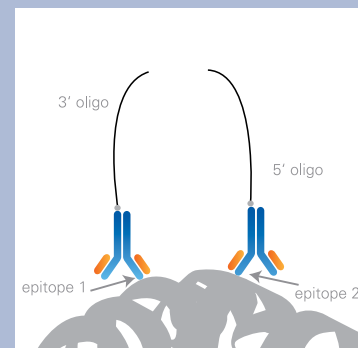
Use crude cell lysates directly; no protein purification is needed.

Cultured cell samples:

- **Protein Quant Sample Lysis Kit**
- **Protein Expression Sample Prep Kit**

Tissue samples:

- **Ambion® PARIS™ Kit**



Binding 60 min*

Combine sample lysate and TaqMan® Protein Assay. Antibodies bind target protein, bringing the attached assay probe oligonucleotides into proximity.

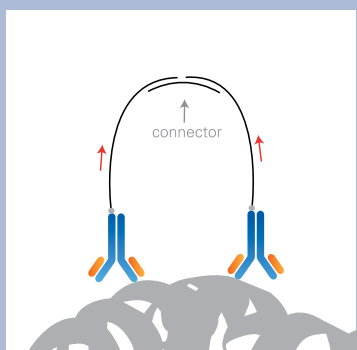
- **TaqMan® Protein Assays Open Kit: make your own assays**
- **TaqMan® Protein Expression Assays Kit: ready-made assays**

*Typical time required

Turn your polyclonal antibody or matched antibody pair into a TaqMan® Protein Assay

Using the TaqMan® Protein Assays Open Kit, you can make assays with either affinity-purified polyclonal antibody preparations or matched antibody pairs specific for unique epitopes on the protein of interest. In many cases, antibody pairs that function well in ELISAs can

be used to make a TaqMan® Protein Assay, providing a homogeneous, liquid-phase testing environment. The TaqMan® Protein Assays Open Kit can also be used to make assays for *in vitro* protein-protein interaction experiments.

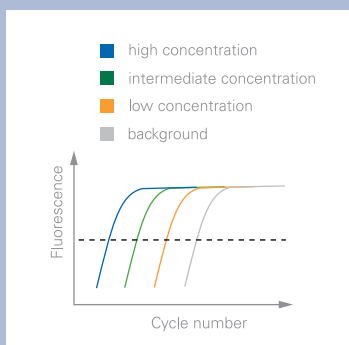


Ligation/ inactivation

25 min*

Assay probe oligonucleotides hybridize to a "connector" oligonucleotide, aligning them so that they can be ligated using DNA ligase. Subsequent protease treatment inactivates the ligase.

- TaqMan® Protein Assays Core Reagents Kit with Master Mix

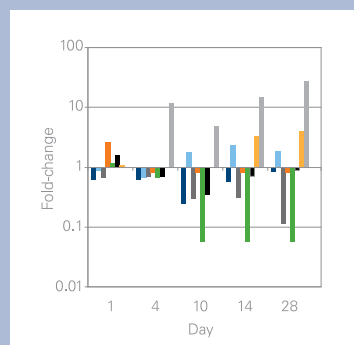


TaqMan® Fast real-time PCR

40 min*

Amplification and detection of the ligation product using a Universal TaqMan® Assay (PCR primer/TaqMan® probe set).

- Any real-time PCR Instrument



Data analysis

Determine relative quantities of proteins and correlate protein expression with mRNA and/or microRNA on a single analysis platform.

- ProteinAssist™ Software

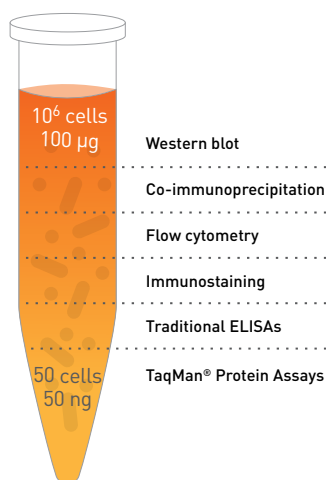
Quantitative protein analysis from limited samples

Whether you are a veteran protein researcher looking for a simpler, more quantitative workflow, or an experienced real-time PCR user seeking to take your gene expression experiments to the protein level, TaqMan® Protein Assay products provide a novel, enabling approach for the most sensitive quantitative protein analysis.

Small samples? No problem.

TaqMan® Protein Assays use 10–500-fold less sample input than traditional protein analysis methods. The typical input sample range is 5–500 cells or tissue lysate containing 1–100 ng total protein.

Typical input quantity



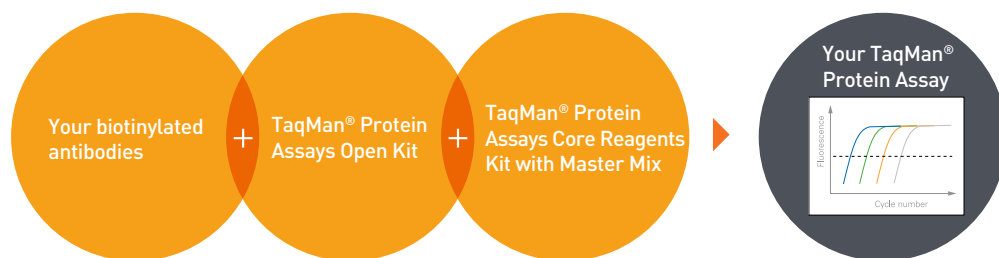
Fast, streamlined assay development and sample analysis

Start with biotinylated, paired antibodies or an affinity-purified polyclonal antibody preparation, and make a TaqMan® Protein Assay for your protein of interest in about 90 minutes using the TaqMan® Protein Assays Open Kit. If you're working with stem cells, one of our predesigned assays for pluripotency markers may suit your needs (see the back cover for a list).

Once you've built your assay, our optimized reagents make it simple to detect your protein(s) with confidence using a streamlined universal protocol.

Data in under 4 hours

We designed TaqMan® Protein Assays to be used with crude cell and tissue lysates, and optimized the system to maximize sensitivity and specificity in measuring protein expression from small samples. Everything takes place in solution. With the homogeneous assay format, there are no washing or purification steps. The simple, rapid protocol can typically be completed in less than 4 hours, including thermal cycling.

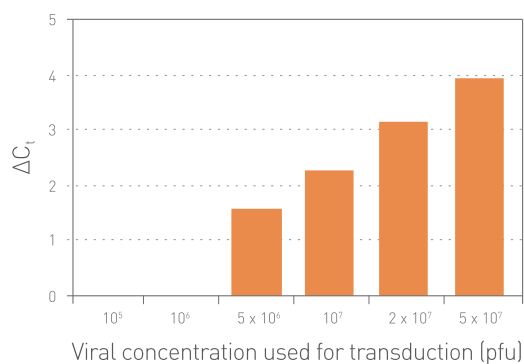


Sensitive, quantitative results

Western blot



TaqMan® Protein Assay

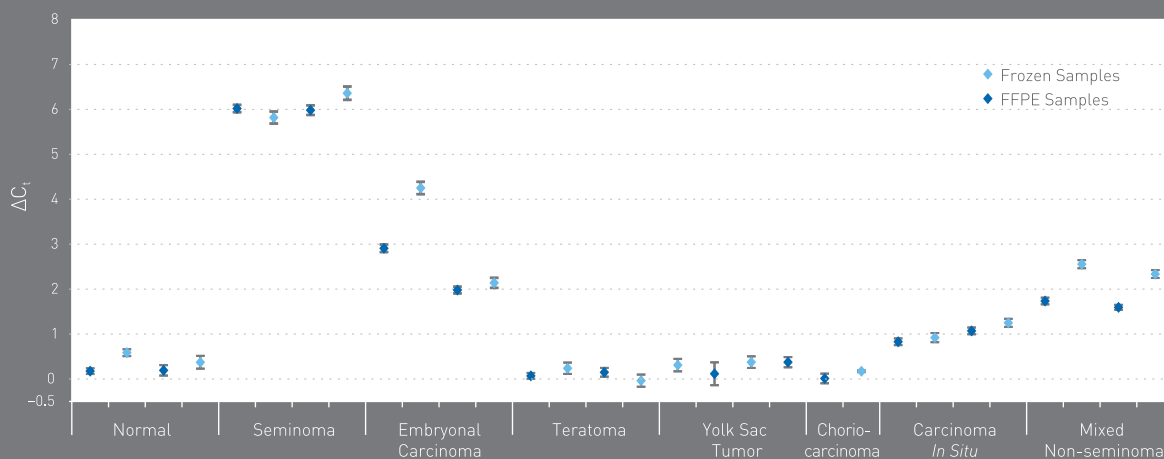


Easily obtain quantitative data without the tedium and hassles of microscopic imaging/counting or running and blotting gels. In addition, data obtained using TaqMan® Protein Assays allows for more accurate protein quantitation, with less subjective analysis than many other methods so that you can quickly and accurately identify fold-change with confidence.

Expression data from TaqMan® Protein Assays is more sensitive and easier to interpret than western blot data. Human dermal fibroblasts (~160,000 cells) were transduced with the indicated concentrations of virus (pfu) expressing the OCT4 gene. Forty-eight hours after transduction, cells were harvested and subjected to analysis using either western blot or a TaqMan® Protein Assay. For the TaqMan® Protein Assay (hOCT3/4), lysate from 500 cells was used for each assay. For western blot analysis, lysate from $\sim 2 \times 10^5$ cells was used for each lane. Protein was detected using an Oct4 antibody and Invitrogen™ WesternBreeze® Chromogenic Western Blot Immunodetection.

TaqMan® Protein Assays can be used to analyze frozen and FFPE tissue samples

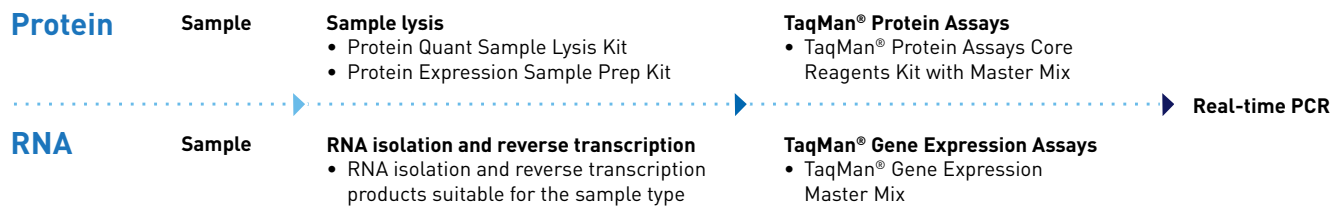
Duplicate matched snap-frozen and formalin-fixed paraffin-embedded (FFPE) samples (10 μ m sections) from normal and cancerous tissues were analyzed using TaqMan® Protein Assays to demonstrate their capability in quantitating protein expression in FFPE samples. Crude protein extracts were prepared from samples using a standard procedure. Extracts were split into triplicates containing 640 ng total protein for analysis using the TaqMan® Protein Assay Kit (hOCT3/4).



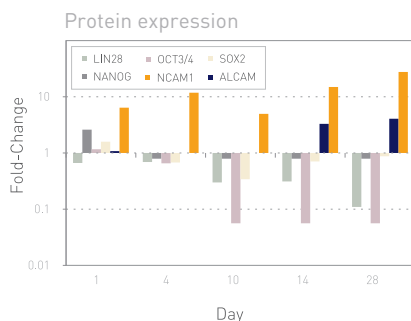
Get more from your real-time PCR workflow

Get more information from your real-time PCR experiments. TaqMan® Protein Assays help you to advance your research quickly by enabling you to detect and quantitate protein, mRNA, and noncoding RNA—including microRNA—all on the same analytical platform.

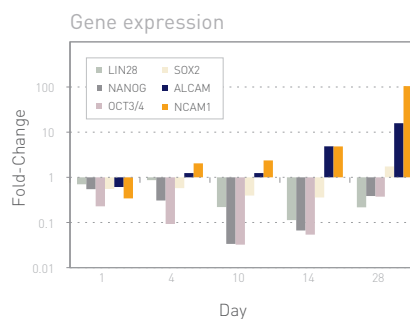
Correlate mRNA and protein expression



Follow your gene expression experiments from start to finish by directly correlating relative RNA quantities and associated protein levels.

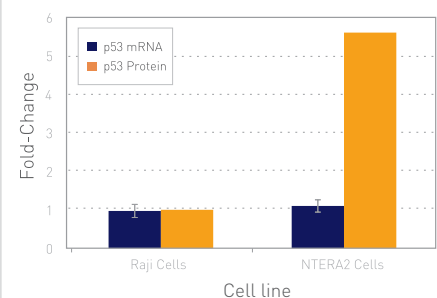


Samples were treated with retinoic acid (RA) and analyzed in parallel for changes in the relative expression of 4 stem cell pluripotency markers and 2 differentiation markers (NCAM1 and ALCAM). RA induction causes the NTERA2 cells to differentiate and develop into neurons over the course of several weeks. NTERA2 cell lysates were prepared with the Protein Expression Sample Preparation Kit; in parallel, RNA was isolated from a portion



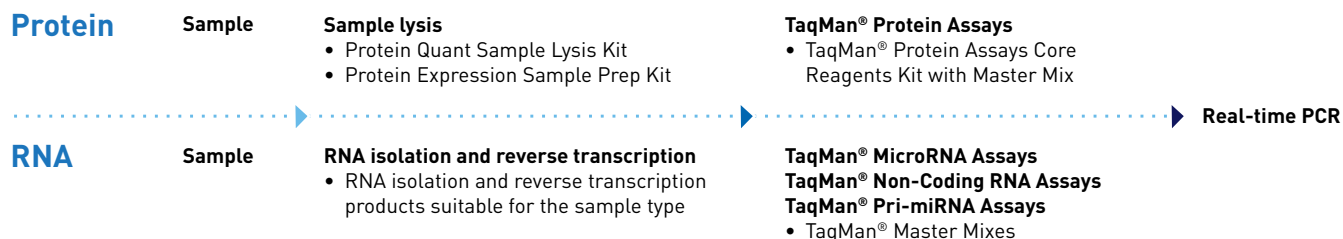
of the samples using the Ambion® PARIS™ and TURBO DNA-free™ Kits. The samples were analyzed for target protein and mRNA transcripts using TaqMan® Assays for protein expression and gene expression, respectively. Real-time PCR was performed on an Applied Biosystems® StepOnePlus™ Real-Time PCR System. The results clearly demonstrate the power of this dual approach for correlating changes in protein expression relative to mRNA levels.

Protein levels do not always correlate with mRNA levels; find out for sure.



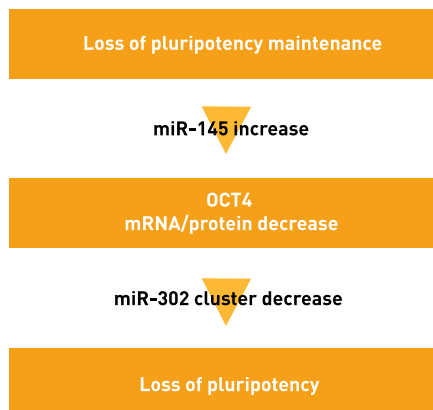
In this model system, genotoxic stress is induced in two cell lines by UV irradiation. Raji cells are more tolerant, but UV irradiation on NTERA2 cells causes apoptosis and later, cell death, in a process that involves p53, a tumor suppressor gene. Compared to cells that were not UV-treated, NTERA2 cells exhibited a nearly 6-fold increase in p53 protein levels after high-dose UV irradiation. Interestingly, p53 mRNA levels were nearly unchanged. This indicates that the mode of p53 protein upregulation was unrelated to mRNA expression (post-translational processes evidently control p53 levels in irradiated cells).

Correlate noncoding RNA and protein expression

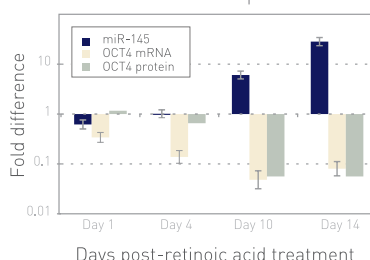


Follow the pathway with TaqMan® Assays for protein, mRNA, and microRNA.

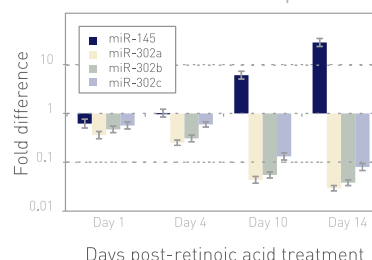
Loss of Pluripotency: miR-145 and OCT4



Increased miR-145 expression/
decreased OCT4 expression



Increased miR-145 expression/
decreased miR-302 expression



In early neuronal development, miR-145 is an important regulator of OCT4 mRNA and protein levels. In this retinoic acid-induced differentiation study, the dramatic increase in the relative expression level

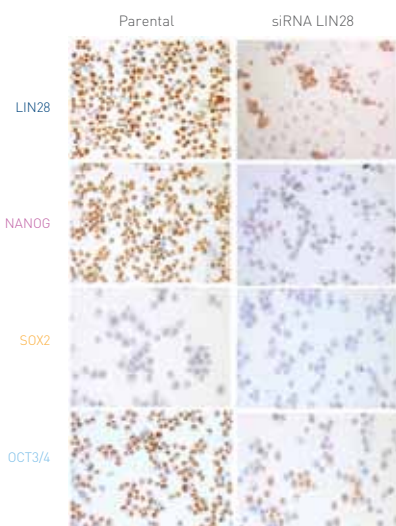
of miR-145 is contrasted to a decrease in the level of OCT4 mRNA, and more importantly, OCT4 protein. A downstream impact of OCT4 protein loss is a noted decrease in the level of miR-302 miRNA.

Validate siRNA knockdown at the protein level

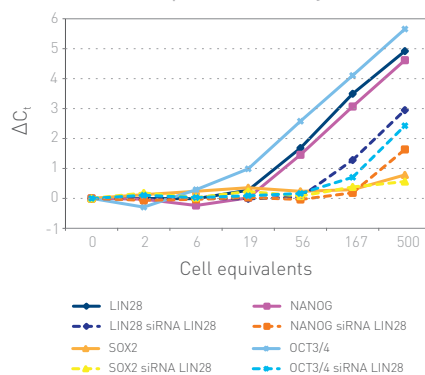


Get quantitative knockdown data for protein using the same instrument platform you're using to show mRNA knockdown.

Immunohistochemistry



Protein Expression Assay



Both immunohistochemistry (IHC) and TaqMan® Protein Assay analyses were performed on cells from a human seminoma cell line, TCam2, to investigate the levels of hLIN28, hNANOG, hSOX2, and hOCT3/4 proteins, 4 common biomarkers of the pluripotent state. The 4 proteins were present in different concentrations, and were easily quantitated based on data obtained using the TaqMan® Protein Assays. Furthermore, quantitation based on protein assay data was more accurate than trying to quantitate expression levels from the IHC data. The effect of transfecting the TCam2 cells with a LIN28 siRNA on these 4 pluripotent markers was also examined. After siRNA transfection, a reduction in LIN28 expression as well as NANOG and OCT3/4 protein expression was seen.

Get more from your antibodies

TaqMan® Protein Assays expand the utility of your affinity-purified polyclonal antibody preparations or matched antibody pairs, enabling you to perform experiments that were not possible before.

Skip the washing steps: get better sensitivity and a homogeneous assay format

Commercially-available antibodies were used to prepare TaqMan® Protein Assays with the TaqMan® Protein Assays Open Kit. The resulting assays were evaluated using 2 µL samples to determine their limit of target detection (LOD). The table compares the LOD reported by the antibody manufacturers (when available) to that obtained using TaqMan® Protein Assays.

TaqMan® Protein Assays often provide better sensitivity than traditional antibody assays, and they offer a convenient homogeneous format.

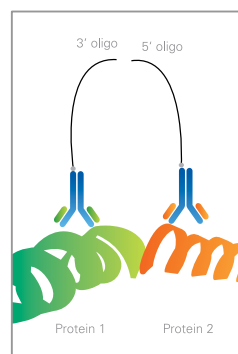
			TaqMan® Protein Assay	
Target	Antibody type	Vendor-published LOD*	LOD (pg/mL)	LOD/assay
TNFR1	Pair	50 pg/mL	16	32 fg/well
DCN	Pair	30–50 pg/mL	13	26 fg/well
Human CSTD	Polyclonal	0.3 ng/well**	38	76 fg/well
Mouse CSTD	Polyclonal	NA	1,600	3.2 pg/well
SCF	Polyclonal	0.3 ng/well**	27	54 fg/well
CD117	Polyclonal	NA	14	28 fg/well
p53	Polyclonal	NA	130	260 fg/well
Pro-CASP3	Polyclonal	NA	400	800 fg/well
CASP8	Polyclonal	NA	1,400	2.8 pg/well
CDH1/E-cadherin	Polyclonal	1.0 ng/well**	4.0	8.0 fg/well

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

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

Directly measure interacting proteins in lysates obtained from only a few thousand cells

TaqMan® Protein Assay for interacting proteins

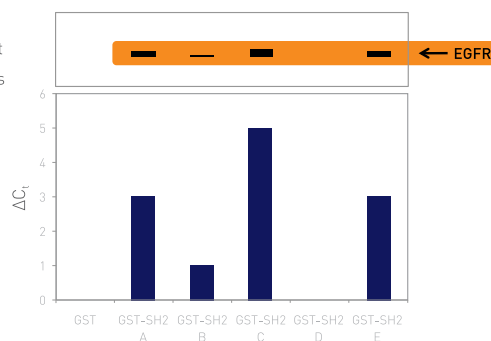


Far western
25–50 µg input
Time: 1–2 days

TaqMan® Protein Assay
<1 µg input
Time: 4 hr

Protein–protein interactions drive the assembly of signaling complexes that transduce signals to downstream effectors. For example, in tyrosine kinase pathways, specific phosphorylation events create binding sites for phosphotyrosine-recognizing modules such as SH2 domains on

TaqMan® Protein Assay data is quantitative and clear



interacting proteins. Here we compare data from adapted western blot (far-western) and TaqMan® Protein Assay approaches for analyzing the interaction of epidermal growth factor (EGF)-stimulated EGF receptor (EGFR) using various glutathione S-transferase (GST)-SH2 fusion proteins in

A431 cell lysates. For the far-western blot, crude protein extract was western blotted and incubated with GST-SH2 fusion proteins. Protein binding was detected using a light reaction catalyzed by horseradish peroxidase-conjugated anti-GST secondary antibody.

The graph shows data obtained using a TaqMan® Protein Assay prepared using the TaqMan® Protein Assays Open Kit and antibodies specific for EGFR (for probe A) and GST (for probe B). When the assay probes targeting each protein come into proximity (as when their cognate proteins interact), the sample yields a positive signal. The data show increased binding intensity to the activated receptor—comparable to results from the far-western technique (fusion proteins A, C, and E). However, the TaqMan® Protein Assay approach requires far less sample and time to obtain results. In addition, the data from TaqMan® Protein Assays is quantitative rather than qualitative.



The TaqMan® Assays QPCR Guarantee*

Life Technologies stands behind every predesigned TaqMan® Assay you buy from us. We are committed to helping you achieve your research goals and strongly believe that our predesigned TaqMan® probe and primer sets are the benchmark in the qPCR industry.

We want you to be happy with your purchase and confident in the genomic tools Life Technologies provides—therefore, we guarantee every TaqMan® Assay in terms of:

- **Quality**—high-quality manufacturing for reproducible results from lot-to-lot
- **Performance**—superior sensitivity, specificity, and accuracy
- **Content**—the largest collection of qPCR primer and probe sets using the world's best and most extensively validated assay design pipeline
- **Results**—data you can trust

If you are not satisfied with the performance of a predesigned TaqMan® Assay, we'll replace it at no cost, or credit your account. For more information and full terms and conditions of the guarantee, go to www.appliedbiosystems.com/taqmanguarantee.

* The TaqMan® Assays QPCR Guarantee covers predesigned TaqMan® Protein Assays only; it does not apply to kits used to make these assays.

Sample prep for TaqMan® Protein Assays

Minimal sample preparation is needed for analysis using TaqMan® Protein Assays. We offer two products for sample preparation from cultured cells developed specifically for use with TaqMan® Protein Assays. Both enable a gentle, one-step cell lysis using a buffered non-ionic detergent; the resulting crude lysates can be directly mixed with the paired assay probes from any TaqMan® Protein Assay for the target binding step.

- **Protein Expression Sample Prep Kit:** The cell lysis reagent contains carrier protein, so cells must be counted prior to lysis in order to obtain relative quantitative data from experiments.
- **Protein Quant Sample Lysis Kit:** The cell lysis reagent provided with this kit does not contain carrier protein, so quantitative results can be obtained either by counting cells before lysis or by normalizing to the total protein content of samples.

Tissue samples can be disrupted in the Cell Disruption Buffer provided with the **Ambion® PARIS™ Kit** (Part no. AM1921).

Free software for data analysis

ProteinAssist™ Software enables relative quantitative analysis of multiple plate studies (100+), and includes a fold-change graph with multi-level sample grouping and a heatmap feature to summarize large datasets.

Ordering information

Description	Size	Part no.
TaqMan® Protein Assays Open Kit		
(Use these kits to make TaqMan® Protein Assays from your own biotinylated polyclonal antibody preparation or matched antibody pair.)		
TaqMan® Protein Assays Open Kit (Contains the TaqMan® Protein Assays Oligo Probe and Buffer Kits)	4,000 rxns	4453745
TaqMan® Protein Assays Oligo Probe Kit	4,000 rxns	4448549
TaqMan® Protein Assays Buffer Kit	4,000 rxns	4448571
Kits for running TaqMan® Protein Assays (required)		
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 Assays Fast Master Mix	100 rxns	4400088
TaqMan® Protein Assays Core Reagents Base Kit	100 rxns	4405460
Sample preparation kits		
Protein Quant Sample Lysis Kit	25 mL	4448536
Protein Expression Sample Prep Kit	1,000 rxns	4405443
Lysate control kits		
Protein Expression Lysate Control Kit (Raji)	100 rxns	4405448
Protein Expression Lysate Control Kit (NTERA2)	100 rxns	4405454
TaqMan® Protein Assays (Ready-to-go assays for proteins involved in stem cell research)		
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

For more information, go to www.appliedbiosystems.com/proteinassays

Life Technologies offers a breadth of products | DNA | RNA | PROTEIN | CELL CULTURE | INSTRUMENTS

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