QuantiSir™ General Gene Knockdown Quantification Kit

Base Catalog # P-5001

PLEASE READ THIS ENTIRE USER GUIDE BEFORE USE

The *QuantiSir*™ General Gene Knockdown Quantification Kit is suitable for quantifying gene knockdown caused by siRNA or antisense oligonucleotides using mammalian tissue and cell extracts.

The *QuantiSir*™ General Gene Knockdown Quantification Kit offers a flexible choice of antibodies of interest. For the best results, the monoclonal antibodies should be used as a capture antibody with this kit. The secondary antibody should be labeled with HRP.

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KIT CONTENTS

Components	48 assays P-5001-48	96 assays P-5001-96
Q1 (Extraction Buffer)	6 ml	12 ml
Q2 (10X Wash Buffer)	14 ml	28 ml
Q3 (Protein Capture Buffer)	0.5 ml	1 ml
Q4 (Blocking Buffer)	10 ml	20 ml
Q5 (Antibody Buffer)	6 ml	12 ml
Q6 (Developing Solution)	5 ml	10 ml
Q7 (Stop Solution)	3 ml	6 ml
GAPDH Control Antibody*	10 µl	20 µl
Detection Antibody*	6 µl	12 µl
8-Well Assay Strips (with Frame)	6	12

** For maximum recovery of the products, centrifuge the original vial after thawing prior to opening the cap.*

SHIPPING & STORAGE

The kit is shipped in two parts, one part at ambient room temperature, and the second part on frozen ice packs at 4°C.

Upon receipt: (1) Store the **Detection Antibody** at –20°C; (2) Store **Q2**, **Q4**, **Q6**, **GAPDH Control Antibody**, and **8-Well Assay Strips** at 4°C away from light; (3) Store **all other components** at room temperature. The kit is stable for up to 6 months from the shipment date, when stored properly.

Note: *Check if wash buffer, **Q2**, contains salt precipitates before using. If so, warm (at room temperature or 37°C) and shake the buffer until the salts are re-dissolved.*

MATERIALS REQUIRED BUT NOT SUPPLIED

- ☐ Centrifuge
- ☐ Orbital shaker
- ☐ Pipettes and pipette tips
- ☐ 15 conical tubes
- ☐ 1.5 ml microcentrifuge tubes
- ☐ PBS
- ☐ Distilled water
- ☐ Primary antibody of interest
- ☐ Secondary antibody

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GENERAL PRODUCT INFORMATION

Quality Control: EpigenTek guarantees the performance of all products in the manner described in our product instructions.

Product Updates: EpigenTek reserves the right to change or modify any product to enhance its performance and design. The information in this User Guide is subject to change at any time without notice. Be sure to use the latest User Guide for this kit which can be accessed online at www.epigentek.com/datasheet.

Usage Limitation: The *QuantiSir*™ General Gene Knockdown Quantification Kit is for research use only and is not intended for diagnostic or therapeutic application.

Intellectual Property: The *QuantiSir*™ kits and methods of use contain proprietary technologies by EpigenTek. *QuantiSir*™ is a trademark of EpigenTek Group Inc.

A BRIEF OVERVIEW

Targeted gene knockdown using small interfering RNA (siRNA) or antisense oligonucleotide has been valuable technology for studying gene function. Gene knockdown leads to reduction in mRNA and subsequently protein expression. It can often be verified at mRNA level by Northern blot or quantitative RT-PCR. However, decrease in the amount of a specific mRNA does not typically correlate well with protein levels present in the cell. Gene knockdown can also be measured at the protein level with Western blot. Western blot analysis is the most comprehensive way of showing that expression of the target gene has been down-regulated. However, this method, while sensitive, often lacks the ability to discriminate between samples in which the differences in protein levels are minimal. It is also limited in its application to high-throughput analysis. To address these problems, EpigenTek has developed the *QuantiSir*™ gene knockdown assay system to quantify gene knockdown induced by siRNA or antisense oligonucleotide at the protein level in cultured cells or tissues. The assay system includes a general gene knockdown assay kit and the specific gene knockdown assay kits, and allows directly measuring a specific protein level in cell lysates. The kit has the following features:

- Quick and efficient procedure – completion of the entire assay needs only 4 hours.
- Innovative colorimetric assay without the need for radioactivity, electrophoresis, or chromatography.
- The convenient internal control is included for standardization and normalization of target gene knockdown effect.
- Strip microplate format makes the assay flexible: manual or high throughput.
- Simple, reliable, and consistent assay conditions.

PRINCIPLE & PROCEDURE

The *QuantiSir*™ General Gene Knockdown Quantification Kit is specifically designed for quantifying gene knockdown induced by siRNA or antisense oligonucleotide at the protein level in the cultured cells or tissues. In the assay, the cell lysates containing the targeted protein are stably

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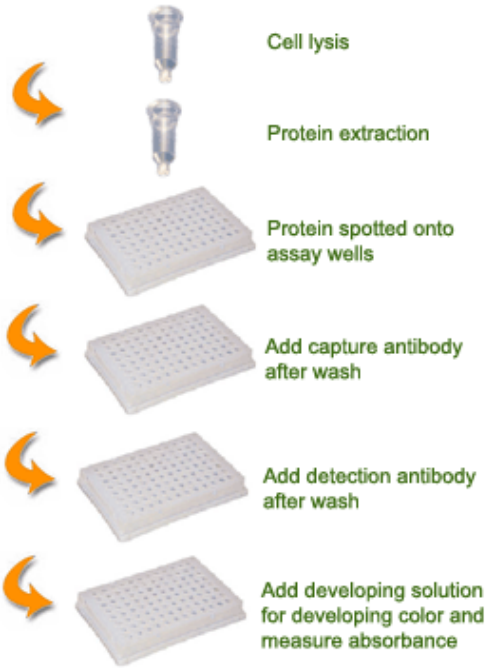
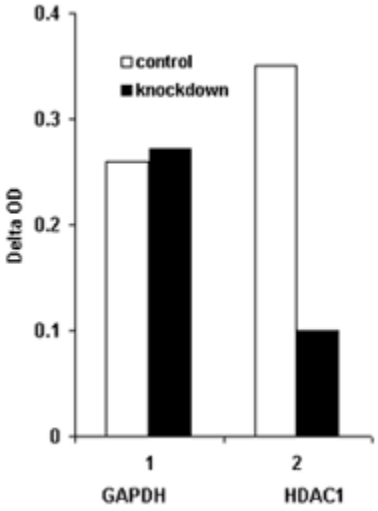
spotted on the specifically treated microwells with unique protein capture buffer. The spotted protein can then be recognized with the target-specific antibody and colorimetrically measured through detection antibody-chromogen reaction system. GAPDH expression as the internal control can be used for normalizing the effect of target gene knockdown.



Schematic Procedure for Using the *QuantiSir*™ General Gene Knockdown Quantification Kit



Quantification of HDAC1 knockdown. MCF-7 cells were treated or untreated with HDAC1 siRNA. Protein extracts were prepared and used for detection of HDAC1 protein level.

PROTOCOL

Protein Extraction

For Adherent Cells:

1. Grow cells (treated or untreated) to 70-80% confluency in a 12-well or 6- well plate; trypsinize, and collect cells into a 15 ml tube.
2. Centrifuge the cells at 1000 rpm for 5 minutes and discard the supernatant. Add 1 ml of PBS into the cell pellet, suspend and transfer cells into a 1.5 ml vial. Pellet cells again by centrifuging at 1000 rpm for 5 minutes.
3. Remove supernatant as much as possible and add **Q1** (40 µl/well for 12- well plate and 100 µl/well for 6-well plate) to re-suspend cell pellet, and then vortex and incubate on ice for 10 minutes.
4. Pellet cell debris by centrifuging at 12,000 rpm for 10 minutes at 4°C. Transfer the supernatant to a new vial. At this stage the supernatant can be used immediately or stored at –80°C.

Note: *For 96-well plate cultures,* **Q1** *can be directly added into the wells in 5 µl/well and incubated at room temperature for 5 minutes to lyse cells. The lysed cell solution is transferred to a 0.5 ml vial and centrifuged at 12,000 rpm for 10 minutes. Supernatant is transferred to a new 0.5 ml vial for storage or to the strip well for assay (see below).*

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For Suspension Cells:

1. Collect cells (treated or untreated) into a 15 ml conical tube. Count cells in a hemacytometer.
2. Centrifuge the cells at 1000 rpm for 5 minutes and discard the supernatant. Add 1 ml of PBS into the cell pellet, suspend, and transfer cells into a 1.5 ml vial. Pellet cells again by centrifuging at 1000 rpm for 5 minutes.
3. Remove supernatant as much as possible and add **Q1** (50 µl/1 x 10^6 cells) to re-suspend cell pellet, and then vortex and incubate on ice for 10 minutes.
4. Pellet cell debris by centrifuging at 12,000 rpm for 10 minutes at 4°C. Transfer the supernatant to a new vial. At this stage the supernatant can be immediately used or store at –80°C.

Target Protein Level Detection

1. Determine the number of strip wells required. Leave these strips in the plate frame (remaining unused strips can be placed back in the bag. Seal the bag tightly and store at 4°C). Dilute **Q2** with distilled water (pH 7.2-7.5) at a 1:10 ratio (ex: 1 ml of **Q2** + 9 ml of distilled water).
2. Dilute the protein extract with **Q3** at a 1:1 ratio (ex: 5 µl of **Q3** + 5 µl of protein extracts). Add 10 µl of the *diluted protein extract* into the central area of each strip well. Spread out the solution over the strip well surface by pipetting the solution up and down several times. Incubate the strip wells at 37°C (with no humidity) for 90 minutes to evaporate the solution and dry the wells. For the blank, add 10 µl of **Q3** instead of protein extract.
   Note: *The non-evaporated solution may be gathered along the edges at the bottom of the well. Make sure the well is completely dry by slightly tilting the well and aspirating against the edge with a P-10 or P-20 pipette. If there is still the residue solution, extend incubation time for an additional 15-30 minutes to dry the well.*
3. Add 150 µl of **Q4** to the wells and incubate at 37°C for 30-45 minutes.
4. Aspirate and wash the wells with 150 µl of **diluted Q2** three times.
5. Dilute the **GAPDH Control Antibody** (at a 1:100 ratio) to 1 µg/ml with **Q5**. Also dilute the antibodies specific for the target proteins to the appropriate concentration (0.5-1 µg/ml) with **Q5**. Add 50 µl of the **diluted GAPDH Control Antibody** and your antibodies to the wells separately and incubate at room temperature for 60 minutes on an orbital shaker (50-100 rpm).
6. Aspirate and wash the wells with 150 µl of **diluted Q2** four times.
7. Dilute the **Detection Antibody** (at a 1:1000 ratio) with **Q5**. Add 50 µl of the **diluted Detection Antibody** to the well in which **GAPDH Control Antibody** was added. Also, dilute the secondary antibodies affinity to the capture antibodies with **Q5** to an appropriate concentration (0.1-0.2 µg/ml). And add 50 µl of secondary antibodies into the wells the capture antibodies were added. Incubate at room temperature for 30 minutes
8. Aspirate and wash the wells with 150 µl of the **diluted Q2** five times.
9. Add 100 µl of **Q6** to the wells and incubate at room temperature for 2-10 minutes away from light. Monitor the color development in the sample and control wells (blue).
10. Add 50 µl of **Q7** to the wells and read absorbance on microplate reader at 450 nm.
11. Calculate % target protein level:

$$\text{Protein \%} = \frac{OD_T\,(\text{treated sample} - \text{blank})/OD_C\,(\text{untreated control} - \text{blank})}{OD_T\,(\text{untreated control} - \text{blank})/OD_C\,(\text{treated sample} - \text{blank})} \times 100\%$$

Here OD_T is OD value for the target protein. OD_C is OD value for the GAPDH control.

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TROUBLESHOOTING

No Signal for the Sample

The protein sample is not properly extracted.	Ensure the protein extraction protocol is suitable for your protein sample preparation.
The protein amount is added into well insufficiently.	Ensure extract contains a sufficient amount of proteins.
Reagents are added incorrectly.	Check if reagents are added in the proper order and if any steps of the procedure may have been omitted by mistake.
The well is not completely dried.	Ensure the well is incubated with no humidity and dry before adding the blocking buffer.
The well is incorrectly washed before protein spotting.	Ensure the well is not washed before adding protein extracts.
Incubation time and temperature are incorrect.	Ensure the incubation time and temperature described in the protocol are followed correctly.
Protein extracts are incorrectly stored.	Ensure the protein extracts are stored at –80°C.

High Background Present for the Blank

The well is not washed sufficiently.	Check if wash at each step is performed according to the protocol.
Insufficient antibody dilution.	Increase antibody dilution.
Overdevelopment.	Decrease the development time in Step 9 of "Target Protein Level Detection."

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