EPIGENTEK
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QuantiSir™ Specific Gene Knockdown Quantification Kit For Cell Death/Apoptosis

Base Catalog # P-5004

PLEASE READ THIS ENTIRE USER GUIDE BEFORE USE

The *QuantiSir*™ Specific Gene Knockdown Quantification Kit For Cell Death/Apoptosis is suitable for quantifying gene knockdown caused by siRNA or antisense oligonucleotides using mammalian tissue and cell extracts.

The *QuantiSir*™ Specific Gene Knockdown Quantification Kit For Cell Death/Apoptosis series offers a flexible choice of different kits used for measuring knockdown of 65 common genes related to cell death/apoptosis.

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KIT CONTENTS

Components	96 assays P-5004-96
Q1 (Extraction Buffer)	12 ml
Q2 (10X Wash Buffer)	28 ml
Q3 (Protein Capture Buffer)	1 ml
Q4 (Blocking Buffer)	20 ml
Q5 (Antibody Buffer)	12 ml
Q6 (Developing Solution)	10 ml
Q7 (Stop Solution)	6 ml
GAPDH Control Antibody*	5 µl
Capture Antibody*	10 µl
Detection Antibody*	20 µl
8-Well Assay Strips (with Frame)	12
User Guide	1

**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 **Detection Antibody** at –20°C. Store **Q2**, **Q4**, **Q6**, **GAPDH Control Antibody**, **Capture 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
- ☐ Microplate reader
- ☐ Pipettes and pipette tips
- ☐ 15 conical tubes
- ☐ 1.5 ml microcentrifuge tubes
- ☐ PBS
- ☐ Distilled water

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.

Usage Limitation: The *QuantiSir*™ Specific Gene Knockdown Quantification Kit For Cell Death/Apoptosis 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, 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 downregulated. 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:

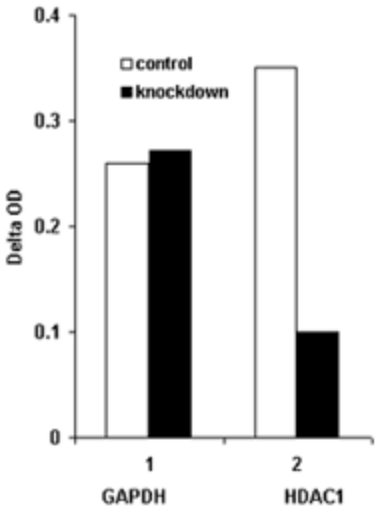
- Quick and efficient procedure. Completion of entire assay needs only 4 hours.
- Innovative colorimetric assay without the need for radioactivity, electrophoresis, or chromatography.
- The convenient internal control is included to correct for the variations for the cell number or protein concentrations.
- Strip microplate format makes the assay flexible: manual or high throughput.
- Simple, reliable, and consistent assay conditions

PRINCIPLE & PROCEDURE

The *QuantiSir*™ Specific Gene Knockdown Quantification Kit For Cell Death/Apoptosis 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 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.

Schematic Procedure for Using the *QuantiSir*™ Specific Gene Knockdown Quantification Kit For Cell Death/Apoptosis



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 12 well or 6 well plate, trypsinize, and collect cells into 15 ml tube.
2. Centrifuge the cells at 1000 rpm for 5 min 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 min.
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, vortex, and incubate on ice for 10 min.
4. Pellet cell debris by centrifuging at 12,000 rpm for 10 min 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 directly be added into the wells in 5 µl/well and incubate at room temperature for 5 min to lyse cells. The lysed cell solution is transferred to a 0.5 ml vial and centrifuge at 12,000 rpm for 10 min. Supernatant is transferred to a new 0.5 ml vial for storage or to the strip well for assay (see below).

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 min 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 min.
3. Remove supernatant as much as possible and add **Q1** (50 µl/ 1 x 10^6 cells) to re-suspend cell pellet, vortex, and incubate on ice for 10 min.
4. Pellet cell debris by centrifuging at 12,000 rpm for 10 min at 4°C. Transfer the supernatant to a new vial. At this stage the supernatant can be used immediately or stored 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.
2. Dilute the protein extract with **Q3** at a 1:1 ratio (ex: add 5 µl of **Q3** to 5 µl of protein extracts). Add 10 µl of the diluted protein extract into 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 min to evaporate the solution and dry the wells). For 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 min to dry the well.*

3. Add 150 µl of **Q4** to the wells and incubate at 37°C for 30-45 min.
4. Aspirate and wash the wells with 150 µl of **diluted Q2** three times.
5. Dilute GAPDH control antibody (at a 1:1000 ratio) to 1 µg/ml with **Q5**. Also dilute the capture antibody (at a 1:500 ratio) to 1 µg/ml with **Q5**. Add 50 µl of the diluted GAPDH control antibody and capture antibody to the wells and incubate at room temperature for 60 min 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 each well. Incubate at room temperature for 30 min.
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 min away from light. Monitor 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.

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 enough amount of proteins.
Reagents are added incorrectly.	Check if reagents are added in 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 block 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 nuclear extracts are stored at –80°C.

High Background Present for the Blank

The well is not washed enough.	Check if wash at each step is performed according to the protocol.
Insufficient antibody dilution.	Increase antibody dilution.
Overdevelopment.	Decrease development time in step 9 of "target protein level detection."

RELATED PRODUCTS

Target	Cat. No.	Target	Cat. No
53BP	5004-53BP	BAP1	5004-BAP1
AIF	5004-AIF	BAP31	5004-BAP31
APAF1	5004-APAF1	Bax	5004-BAX
ARTS	5004-ARTS	BCL-xl	5004-BCLXL
BAD	5004-BAD	BCL-2	5004-BCL2
BAG1	5004-BAG1	BCL-6	5004-BCL6
BAK	5004-BAK	BCL-10	5004-BCL10

BCL-11	5004-BCL11	DR4	5004-DR4
BCL-w	5004-BCLW	DR5	5004-DR5
BID	5004-BID	FADD	5004-FADD
BMF	5004-BMF	FAF1	5004-FAF1
BNIP3	5004-BNIP3	FASL	5004-FASL
B-Raf	5004-BRAF	FLIP	5004-FLIP
CAD	5004-CAD	HIF	5004-HIF
CAS	5004-CAS	ICAD	5004-ICAD
Caspase-1	5004-CASP1	IGFBP3	5004-IGFBP3
Caspase-2	5004-CASP2	IKK-a	5004-IKKA
Caspase-3	5004-CASP3	IKK-b	5004-IKKB
Caspase-4	5004-CASP4	IKK-r	5004-IKKR
Caspase-6	5004-CASP6	PIDD	5004-PIDD
Caspase-7	5004-CASP7	PUMA	5004-PUMA
Caspase-8	5004-CASP8	RAF-1	5004-RAF1
Caspase-9	5004-CASP9	RAIDD	5004-RAIDD
CD-95	5004-CD95	SMAC	5004-SMAC
Cytochrome C	5004-CYTOC	XIAP	5004-XIAP
DAP	5004-DAP	Survivin	5004-SURVIV
DAPK1	5004-DAPK1	TRADD	5004-TRADD
DcR1	5004-DCR1	TRAF2	5004-TRAF2
DcR2	5004-DCR2	TRAIL	5004-TRAIL
DEDD	5004-DEDD	TRAP1	5004-TRAP1
DICE1	5004-DICE1	TOB	5004-TOB
DR3	5004-DR3	TWEAK	5003-TWEAK

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