QuantiSir™ Specific Gene Knockdown Quantification Kit For DNA Damage/Repair

Base Catalog # P-5003

PLEASE READ THIS ENTIRE USER GUIDE BEFORE USE

The *QuantiSir*™ Specific Gene Knockdown Quantification Kit For DNA Damage/Repair 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 DNA Damage/Repair series offers a flexible choice of different kits used for measuring knockdown of 46 common genes related to DNA damage/repair.

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KIT CONTENTS

Components	96 assays P-5003-96
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*	20 µl
Capture Antibody*	50 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; (2) 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.

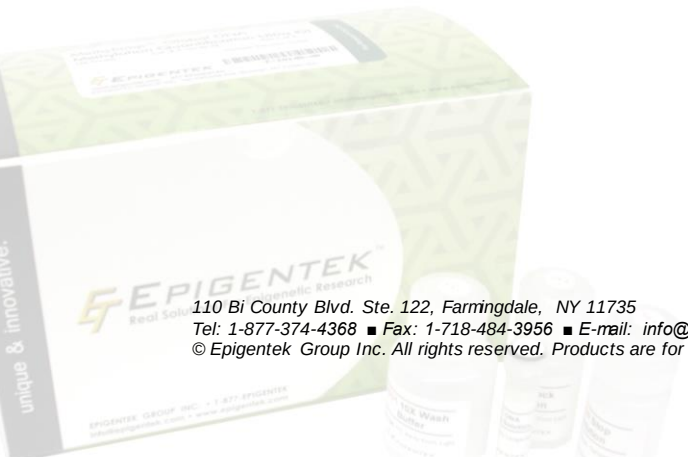
Usage Limitation: The *QuantiSir*™ Specific Gene Knockdown Quantification Kit For DNA Damage/Repair is for research use only and is not intended for diagnostic or therapeutic application.

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 be often 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 be also 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. Completion of entire assay needs only 4 hours.
- Innovative colorimetric assay with no 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.



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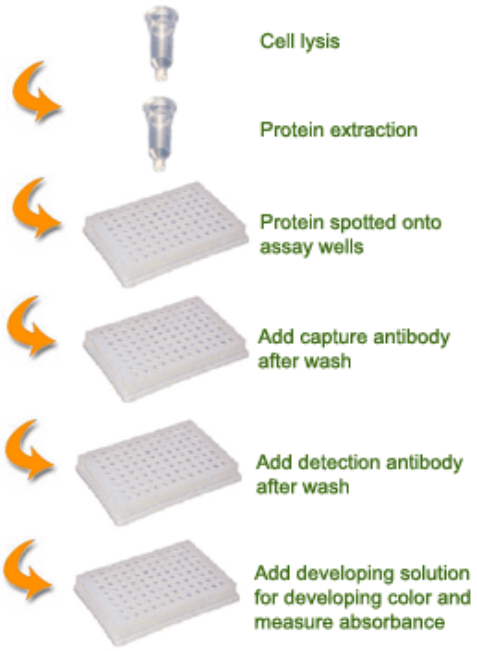
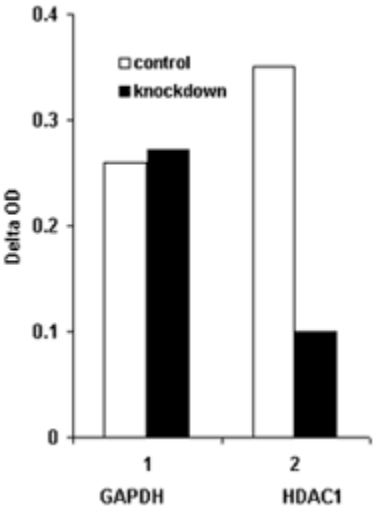
PRINCIPLE & PROCEDURE

The *QuantiSir*™ Specific Gene Knockdown Quantification Kit For DNA Damage/Repair 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 be then 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 DNA Damage/Repair



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 1,000 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 immediately used 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 min to lyse cells. The lysed cell solution is transferred to a 0.5 ml vial and centrifuged 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 1,000 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 immediately used or stored at –80°C.

Target Protein Level Detection

1. Determine the number of the strip wells required. Leave these strips in the plate frame (remaining unused strips can be put 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 1:100 ratio) to 1 µg/ml with **Q5**. Also dilute the capture antibody (at a 1:100-200 ratio) 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 correctly followed.
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
ATM	5003-ATM	PMS2	5003-PMS2
ATR	5003-ATR	PRDX4	5003-PRDX4
APE	5003-APE	Rad1	5003-RAD1
BACH1	5003-BACH1	Rad9	5003-RAD9
CTIP	5003-CTIP	Rad17	5003-RAD17
CRE	5003-CRE	Rad21	5003-RAD21
DDB1	5003-DDB1	Rad50	5003-RAD50
DDB2	5003-DDB2	Rad51	5003-RAD51
DMC1	5003-DMC1	Rad52	5003-RAD52
ERCC1	5003-ERCC1	Rad54	5003-RAD54
ERCC2	5003-ERCC2	RPA	5003-RPA
FANCD2	5003-FANCD2	RPA14	5003-RPA14
GSTP1	5003-GSTP1	RPA32	5003-RPA32
Ku70	5003-KU70	SMC1	5003-SMC1
Ku80	5003-KU80	SOD	5003-SOD
MDR1	5003-MDR1	XPA	5003-XPA
MGMT	5003-MGMT	XPC	5003-XPC
MLH1	5003-MLH1	XPF	5003-XPF
Mre11	5003-MRE11	XPG	5003-XPG
MSH2	5003-MSH2	XRCC1	5003-XRCC1
MSH6	5003-MSH6	XRCC2	5003-XRCC2
Mue81	5003-MUE81	XRCC3	5003-XRCC3
Nm23	5003-NM23	XRCC4	5003-XRCC4

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