DATA SHEET

QuantiSir™ Specific Gene Knockdown Quantification Kit For Transcription Factors

Catalog No. P-5009

User Guide*

*Always use the most updated User Guide included in your current order.

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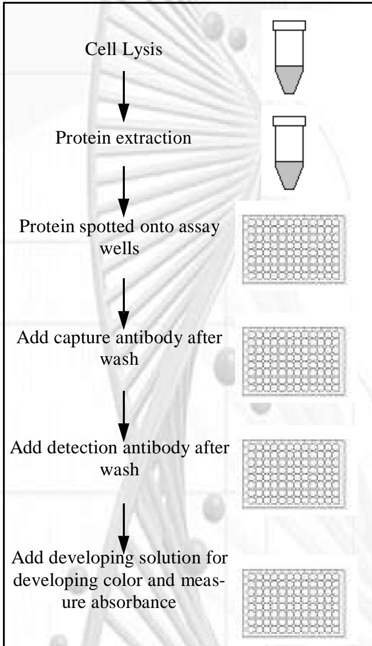
INTRODUCTION

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:

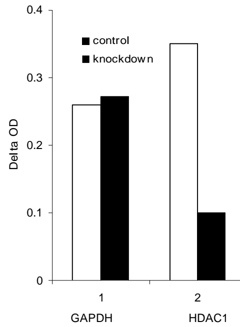
- Quick and efficient. Completion of entire assay needs only 4 hours.
- Innovative colorimetric assay with no need for radioactivity, electrophoresis, and chromatography.
- The internal control is conveniently included to correct for the variations for the cell number or protein concentrations.
- Strip microplate format makes the assay flexible: manual or high throughput.
- Simple, reliable, and consistent assay conditions.

PRINCIPLEANDPROCEDURE

The QuantiSir™ Specific Gene Knockdown Quantification Kit For Transcription Factors 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 Transcription Factors



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.

PRODUCTUSEINFORMATION

The QuantiSir™ Specific Gene Knockdown Quantification Kit For Transcription Factors 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 Transcription Factors series offers a flexible choice of different kits used for measuring knockdown of 47 common genes related to transcription factors.

Epigentek guarantees the performance of all products in the manner described in our product instructions.

Epigentek reserves the right to change or modify any product to enhance its performance and design.

The QuantiSir™ Specific Gene Knockdown Quantification Kit For Transcription Factors is for research use only and is not intended for diagnostic or therapeutic application.

QuantiSir™ is a trademark of Epigentek, Inc.

The QuantiSir™ kits and methods of use are covered by a pending US patent.

KIT CONTENTS

Components	96 assays P-5009-96
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
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* For maximum recovery of the products, centrifuge the original vial after thawing prior to opening the cap.

SHIPPING AND 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 components of the kit should be stable for 6 months 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 redissolved.

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

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 store at –80°C.

Note: For 96 well plate cultures, Q1 can be directly 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 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 store 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 the 1:10 ratios.

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2. Dilute the protein extract with Q3 at the 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 the 1:100 ratios) to 1 µg/ml with Q5. Also dilute the capture antibody (at the 1:100 ratios) 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 the 1:1000 ratios) 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

Problem	Solution
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 some steps of the procedure are 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 is 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

Problem	Solution
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."

ORDERINGINFORMATION

Kit Types for QuantiSir™ Specific Gene Knockdown Quantification Kit For Transcription Factors

Target	Cat. No.	Target	Cat. No.
ABIN1	5009-ABIN1	IRF-1	5009-IRF1
AP1	5009-AP1	Kaiso	5009-KAISO
AP2	5009-AP2	MRG1	5009-MRG1
ARC	5009-ARC	MyoD	5009-MYOD
ATF1	5009-ATF1	NF-1	5009-NF1
ATF-2	5009-ATF2	NFAT2	5009-NFAT2
ATRX	5009-ATRX	Pcaf	5009-PCAF
BLIMP	5009-BLIMP	OCT2	5009-OCT2
BTEB	5009-BTEB	PML	5009-PML
CAF1	5009-CAF1	PU.1	5009-PU.1
CIITA	5009-CIITA	Ring1	5009-RING1
CREB	5009-CREB	SF2/ASF	5009-SF2/ASF
Crel	5009-CREL	SRF	5009-SRF
CREM	5009-CREM	SRY	5009-SRY
CoRes	5009-CORES	TAF	5009-TAF
CtBP	5009-CTBP	TBR1	5009-TBR1
CTCF	5009-CTCF	TFII	5009-TFII
EKLF	5009-EKLF	TFIIB	5009-TFIIB
EGR1	5009-EGR1	TIF-1	5009-TIF1
HBP1	5009-HBP1	TRAP	5009-TRAP
HIF	5009-HIF	TTF	5009-TTF
HMG1	5009-HMG1	WT-1	5009-WT1
HnRNP	5009-HNRNP	YY-1	5009-YY1
HP1	5009-HP1		

Need more components? You can also order parts separately by calling 1-877-374-4368 or e-mailing sales@epigentek.com.

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GENTAUR

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