EpiQuik™ In Vivo Protein Sumoylation Assay Ultra Kit

Base Catalog # P-8003

PLEASE READ THIS ENTIRE USER GUIDE BEFORE USE

Uses: The EpiQuik™ *In Vivo* Protein Sumoylation Assay Ultra Kit is very suitable for measuring *in vivo* protein sumoylation from multiple mammalian cells/tissues including human, mouse, and rat. Nuclear extracts can be prepared by using your own successful method. For your convenience and the best results, EpigenTek offers a nuclear extraction kit (Cat. # OP-0002) optimized for use with this kit. Nuclear extracts can be used immediately or stored at –80°C for future use.

Input Material: Input material is nuclear extracts. The amount of nuclear extracts for each assay can be 1 µg-20 µg with an optimal range of 5 to 10 µg.

Internal Control: The positive control (SUMO- protein) and negative control (non-immune IgG) are provided in this kit for the quantification of sumoylation. Because percentage of sumoylation can vary from tissue to tissue, and from normal and diseased states, it is advised to run replicate samples to ensure that the signal generated is validated.

Precautions: To avoid cross-contamination, carefully pipette the sample or solution into the strip wells. Use aerosol-barrier pipette tips and always change pipette tips between liquid transfers. Wear gloves throughout the entire procedure. In case of contact between gloves and sample, change gloves immediately.

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Component	48 Assays Cat. #P-8003-48	96 Assays Cat. #P-8003-96	Storage Upon Receipt
WB (10X Wash Buffer)	15 ml	30 ml	4°C
BS (Binding Solution)	10 ml	20 ml	4°C
NC (Negative Control, 250 µg/ml)*	10 µl	20 µl	4°C
BB (Blocking Buffer)	10 ml	20 ml	4°C
SAB (SUMO Assay Buffer)	5 ml	10 ml	4°C
PC (Positive Control, 1 µg/µl)*	10 µl	20 µl	-20°C
DA (Detection Antibody, 1000X)*	5 µl	10 µl	-20°C
CD (Color Developer)	5 ml	10 ml	4°C
SS (Stop Solution)	5 ml	10 ml	RT
8-Well Assay Strips (With Frame)	6	12	4°C

** Spin the solution down to the bottom prior to use.*

SHIPPING & STORAGE

The kit is shipped in two parts: the first part at ambient room temperature, and the second part on frozen ice packs at 4°C. Upon receipt: (1) Store **PC** and **DA** at –20°C away from light; (2) Store **WB, BS, NC, BB, SAB**, **CD** and **8-Well Assay Strips** at 4°C away from light; and (3) Store remaining components at room temperature away from light.

All components of the kit are stable for 6 months from the date of shipment, when stored properly.

Note: *(1) Check if **WB** (10X Wash Buffer) contains salt precipitates before use. If so, warm (at room temperature or 37°C) and shake the buffer until the salts are re-dissolved; and (2) check if a blue color is present in **CD** (Color Developer), which would indicate a contamination of the solution and should not be used. To avoid contamination, transfer the amount of **CD** required into a secondary container (tube or vial) before adding **CD** into the assay wells.*

MATERIALS REQUIRED BUT NOT SUPPLIED

- ☐ Adjustable pipette or multiple-channel pipette
- ☐ Multiple-channel pipette reservoirs
- ☐ Aerosol resistant pipette tips
- ☐ Microplate reader capable of reading absorbance at 450 nm
- ☐ 1.5 ml microcentrifuge tubes
- ☐ Incubator for 37°C incubation
- ☐ Antibodies against proteins of interest

- ☐ Nuclear protein samples
- ☐ Parafilm M

GENERAL PRODUCT INFORMATION

Quality Control: Each lot of the EpiQuik™ *In Vivo* Protein Sumoylation Assay Ultra Kit is tested against predetermined specifications to ensure consistent product quality. EpigenTek guarantees the performance of all products in the manner described in our product instructions.

Product Warranty: If this product does not meet your expectations, simply contact our technical support unit or your regional distributor. We also encourage you to contact us if you have any suggestions about product performance or new applications and techniques.

Safety: Suitable lab coat, disposable gloves, and proper eye protection are required when working with this product.

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 EpiQuik™ *In Vivo* Protein Sumoylation Assay Ultra Kit is for research use only and is not intended for diagnostic or therapeutic application.

Intellectual Property: The EpiQuik™ *In Vivo* Protein Sumoylation Assay Ultra Kit and methods of use contain proprietary technologies by EpigenTek. EpiQuik™ is a trademark of EpigenTek Group Inc.

A BRIEF OVERVIEW

Sumoylation is a post-translational modification involved in various cellular processes, such as nuclear-cytosolic transport, transcriptional regulation, apoptosis, protein stability, response to stress, and progression through the cell cycle.

SUMO proteins are similar to ubiquitin. There are 3 confirmed SUMO isoforms in humans: SUMO-1, SUMO-2, and SUMO-3. SUMO-2/3 show a high degree of similarity to each other and are distinct from SUMO-1. Sumoylation is directed by an enzymatic cascade analogous to that involved in ubiquitination. Sumoylation of target proteins *in vivo* has been shown to cause a number of different outcomes, including altered localization and binding partners. In many cases, sumoylation of transcriptional regulators correlates with inhibition of transcription. Most sumoylated proteins contain the tetrapeptide consensus motif Ψ-K-x-D/E where Ψ is a hydrophobic residue, K is the lysine conjugated to SUMO, x is any amino acid (aa), and D or E is an acidic residue. Thus, detection of *in vivo* protein sumoylation (SUMO conjugation) would provide useful information for understanding SUMO modification that emerges as an important control mechanism regulating the activity of many nuclear proteins.

There are few methods currently available for measuring *in vivo* protein sumoylation. The EpiQuik™ *In Vivo* Universal Protein Sumoylation Assay Kit addresses this problem and uses a proprietary and unique procedure to measure *in vivo* protein sumoylation. EpigenTek continues to innovate with the

development of EpiQuik™ *In Vivo* Protein Sumoylation Assay Ultra Kit, which allows increased detection sensitivity and assay convenience. The ultra kit has the following features:

- Fast procedure, which can be finished within 5 hours.
- Direct colorimetric assay without the need for affinity chromatography and Western blotting.
- Flexible choice of antibody of interest allows the detection of sumoylation of multiple target proteins simultaneously.
- Use of optimized detection antibody eliminates the step for detection solution preparation, increasing detection sensitivity and assay convenience.
- The positive control (SUMO protein) and negative control (un-sumoylated non-immune IgG protein) allows protein sumoylation to be quantified.
- Strip microplate format makes the assay flexible: manual or high throughput analysis.
- Reliable and consistent assay conditions.

PRINCIPLE & PROCEDURE

The EpiQuik™ *In Vivo* Protein Sumoylation Assay Ultra Kit is designed for measuring sumoylation of the targeted proteins. Sumoylation of the targeted proteins is indicated by SUMO conjugated to these proteins. In an assay with this kit, the antibodies specific to the targeted proteins are stably bound to the strip wells and the targeted proteins are captured by these antibodies. Sumoylation of the targeted proteins are detected by recognition of SUMO conjugated to these proteins with an anti-SUMO antibody. The ratio or intensity of the sumoylation, which is proportional to the conjugated SUMO amount, can be quantified through the signal report-color development system.

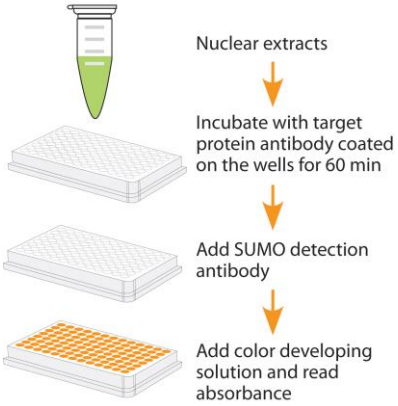

Fig 1. Schematic Procedure for Using the EpiQuik™ *In Vivo* Protein Sumoylation Assay Ultra Kit

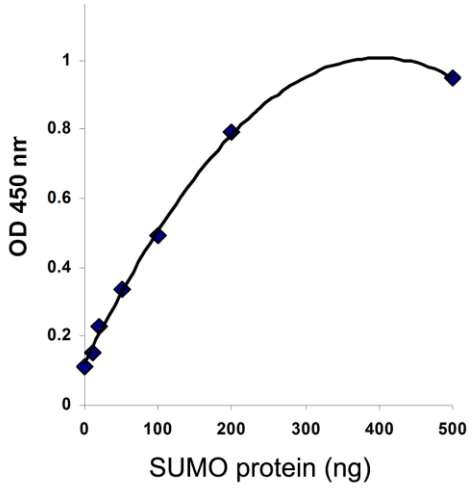

Fig 2. Illustrated standard curve generated with sumo protein control.

PROTOCOL

For the best results, please read the protocol in its entirety prior to starting your experiment.

Starting Materials

Input Amount: The amount of nuclear extracts for each assay can be between 1 and 20 µg with an optimal range of 5 to 10 µg.

Nuclear Extraction: You can use your method of choice for preparing nuclear extracts from the treated and untreated samples. EpigenTek also offers a nuclear extraction kit (Cat # OP-0002) optimized for use with this kit.

Nuclear extracts should be stored in aliquots at –80°C until use.

1. Working Buffer and Solution Preparation

a. Prepare **Diluted WB**:

48-Assay Kit: Add 13 ml of **WB** to 117 ml of distilled water and adjust pH to 7.2-7.5.

96-Assay Kit: Add 26 ml of **WB** to 234 ml of distilled water and adjust pH to 7.2-7.5.

This **Diluted WB** can now be stored at 4°C for up to six months.

b. Prepare **Diluted DA** (Detection Antibody) Solution:

Dilute **DA** with **Diluted WB** at a ratio of 1:1000 (i.e., add 1 µl of **DA** to 1000 µl of **Diluted WB**). 50 µl of **Diluted DA** will be required for each assay well.

c. Prepare diluted antibodies that are specific for the protein of interest. The antibodies should be IP-grade:

Dilute the antibodies to 2 µg/ml with **BS** (Binding Solution). 100 µl of diluted antibodies are required for each sample well.

d. Prepare **Diluted Positive Control** for Standard Curve:

Suggested Standard Curve Preparation: First, dilute **PC** (Positive Control) with **BS** to 250 ng/µl by adding 5 µl of **PC** to 15 µl of **BS**. Then, further prepare five concentrations by combining the 250 ng/µl **Diluted PC** with **BS** into final concentrations of 5, 10, 25, 50, 100, and 250 ng/µl according to the following dilution chart:

Tube	PC (250ng/µl)	BS	Resulting PC Concentration
1	1.0 µl	49.0 µl	5 ng/µl
2	1.0 µl	24.0 µl	10 ng/µl
3	1.0 µl	9.0 µl	25 ng/µl
4	1.0 µl	4.0 µl	50 ng/µl
5	2.0 µl	3.0 µl	100 ng/µl
6	5.0 µl	0.0 µl	250 ng/µl

Note: *Keep each of the diluted solutions except* ***Diluted WB*** *on ice until use. Any remaining diluted solutions other than* ***Diluted WB*** *should be discarded if not used within the same day.*

2. Antibody and Control Coating

a. Predetermine the number of strip wells required for your experiment. It is advised to run replicate samples (include blank and positive controls) to ensure that the signal generated is validated. Carefully remove unneeded strip wells from the plate frame and place them back in the bag (seal the bag tightly and store at 4°C).

b. Blank Wells: Add 100 µl of **BS** to each blank well.

c. Standard Control Wells: Add 98 µl of **BS** to each well and then add 2 µl of **Diluted PC** to each standard control well with a minimum of six wells, each at a different concentration between 10 and 500 ng/well (based on the dilution chart in Step 1d; see Table 1 under the "Suggested Strip Well Setup" section as an example).

d. Sample Wells: Add 100 µl of the diluted antibody solution against the protein of interest to each sample well (from Step 1c).

e. Negative Control Well: Add 98 µl of **BS** to each well and then add 2 µl (0.5 µg) of **NC** (Negative Control).

f. Tightly cover strip-well microplate with Parafilm M to avoid evaporation and incubate at 37°C for 90 to 120 min.

g. Remove the solutions from each well. Add 150 µl of **BB** (Blocking Buffer) to the wells and incubate at room temperature for 45 minutes.

h. Remove the **Blocking Buffer** from each well. Wash each well two times with 150 µl of the **Diluted WB** each time.

3. Sumoylated Protein Capture

a. Blank Wells: Add 50 µl of **SAB** (SUMO Assay Buffer).

b. Standard Control Wells: Add 50 µl of **SAB** to each standard well.

c. Sample Wells: Add 50 µl of **SAB** and then add 5-10 µg of nuclear proteins (total nuclear protein volume should be no more than 10 µl).

d. Negative Control Well: Add 50 µl of **SAB** to each negative control well.

e. Tightly cover strip-wells with Parafilm M to avoid evaporation and incubate at 37°C for 60 min.

f. Remove the reaction solution from each well. Wash each well two times with 150 µl of the **Diluted WB** each time.

4. Detection Antibody Binding

a. Add 50 µl of the **Diluted DA** to each well, then cover with Parafilm M or aluminium foil and incubate at room temperature for 60 min.

b. Remove the **Diluted DA** from each well.

c. Wash each well four times with 150 µl of the **Diluted WB** each time.

Note: *Ensure any residual wash buffer in the wells is removed as much as possible at each wash step.*

5. Signal Detection

a. Add 100 µl of **CD** (Color Developer) to each well and incubate at room temperature for 1 to 10 min away from light. Begin monitoring color change in the sample wells and control wells. The **CD** solution will turn blue in the presence of sufficient SUMO products.

b. Add 100 µl of **SS** (Stop Solution) to each well to stop enzyme reaction when color in the positive control wells turns medium blue. The color will change to yellow after adding **SS** and the absorbance should be read on a microplate reader within 2 to 10 min at 450 nm with an optional reference wavelength of 655 nm.

Note: *(1) Most microplate readers have the capability to carry out dual wavelength analysis and will automatically subtract reference wavelength absorbance from the test wavelength absorbance. If your plate reader does not have this capability, the plate can be read twice, once at 450 nm and once at 655 nm. Then, manually subtract the 655 nm ODs from 450 nm ODs; (2) if the strip-well microplate frame does not fit in the microplate reader, transfer the solution to a standard 96-well microplate.*

6. Sumoylation Calculation

Calculate sumoylation of the targeted proteins.

For simple calculation:

$$\% \text{ Sumoylation} = \frac{\text{OD (treated sample – negative control)}}{\text{OD (untreated sample – negative control)}} \times 100\%$$

For accurate calculation:

(1) Plot Delta OD values (positive control OD–Blank OD) versus amount of **PC** added in the wells and determine the slope as delta OD/ng.

(2) Calculate intensity of the conjugated SUMO using the following formula:

$$\text{Sumoylation intensity (ng/mg protein)} = \frac{\text{OD (sample – negative control)}}{\text{slope} \times \text{protein amount added (µg)}} \times 1000$$

SUGGESTED STRIP WELL SETUP

Table 1. Standard Curve Preparation. The suggested strip-well plate setup for standard curve preparation in a 48-assay format (for a 96-assay format, Strips 7 to 12 can be configured as Sample). The controls and samples can be measured in duplicate.

Well #	Strip 1	Strip 2	Strip 3	Strip 4	Strip 5	Strip 6
A	Blank	Blank	Sample	Sample	Sample	Sample
B	PC 10 ng	PC 10 ng	Sample	Sample	Sample	Sample
C	PC 20 ng	PC 20 ng	Sample	Sample	Sample	Sample
D	PC 50 ng	PC 50 ng	Sample	Sample	Sample	Sample
E	PC 100 ng	PC 100 ng	Sample	Sample	Sample	Sample
F	PC 200 ng	PC 200 ng	Sample	Sample	Sample	Sample
G	PC 500 ng	PC 500 ng	Sample	Sample	Sample	Sample
H	NC	NC	Sample	Sample	Sample	Sample

Table 2. Approximate amount of required buffers and solutions for defined assay wells based on the protocol.

Reagents	1 well	1 strip (8 wells)	2 strips (16 wells)	6 strips (48 wells)	12 strips (96 wells)
Diluted WB	2.5 ml	20 ml	40 ml	120 ml	240 ml
BS	120 µl	1000 µl	2000 µl	6000 µl	12000 µl
NC	N/A	2 µl	4 µl	4 µl	8 µl
BB	150 µl	1200 µl	2400 µl	7200 µl	14.400 µl
SAB	50 µl	400 µl	800 µl	2400 µl	4800 µl
PC	N/A	N/A	3 µl (optional)	5 µl	10 µl
Diluted DA	50 µl	400 µl	800 µl	2400 µl	4800 µl
CD	0.1 ml	0.8 ml	1.6 ml	4.8 ml	9.6 ml
SS	0.1 ml	0.8 ml	1.6 ml	4.8 ml	9.6 ml

TROUBLESHOOTING

Problem	Possible Causes	Suggestions
No signal for the sample	Antibodies are not properly coated.	Ensure the concentration of the antibody solution.
	Antibodies are not IP-grade.	Ensure the antibodies can be used for IP.
	The protein sample is not properly extracted.	Ensure the protein extraction protocol is for nuclear protein extraction.
	The protein amount is added into well insufficiently.	Ensure extract contains a sufficient amount of protein.
	Nuclear extracts are stored incorrectly.	Ensure the nuclear extracts are stored at -80°C.
	Reagents are added incorrectly.	Check if reagents are added in order and if some steps of the procedure were omitted by mistake.
	Incubation time and temperature are incorrect.	Ensure the incubation time and temperature described in the protocol are followed correctly.
	Absence of sumoylation.	N/A.
High background present for the negative control background	The negative control wells are contaminated with antibodies.	Ensure only negative control is added.
	The wells are not sufficiently blocked with **BB.**	Increase blocking time to 60-90 minutes.
	The well is not washed sufficiently.	Check if the wash at each step is performed according to the protocol.
	Overdevelopment.	Decrease development time in Step 4a.

RELATED PRODUCTS

Nuclear Extract Preparation
OP-0002 EpiQuik™ Nuclear Extraction Kit