EpiQuik™ In Vivo Universal Protein Sumoylation Assay Kit

Base Catalog # P-8001

PLEASE READ THIS ENTIRE USER GUIDE BEFORE USE

The *EpiQuik™ In Vivo* Universal Protein Sumoylation Assay Kit is very suitable for measuring *in vivo* protein sumoylation from multiple mammalian cells/tissues including human, mouse, and rat.

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KIT CONTENTS

Components	48 assays P-8001-48	96 assays P-8001-96
S1 (10X Wash Buffer)	25 ml	2 x 25 ml
S2 (Binding Buffer)	15 ml	30 ml
S3 (Negative Control, 1 µg/µl)*	120 µl	240 µl
S4 (Blocking Buffer)	10 ml	20 ml
S5 (Sumo Assay Buffer)	2 ml	4 ml
S6 (Sumo Protein, 1 µg/µl)*	10 µl	20 µl
S7 (Sumo Antibody, 1 µg/µl)*	5 µl	10 µl
S8 (Signal Report Solution)*	5 µl	10 µl
S9 (Color Development Solution)	6 ml	12 ml
S10 (Stop Solution)	3 ml	6 ml
8-Well Assay Strip (with Frame)	6	12
8-Well Control Strips	2	3

** 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: the first part at ambient room temperature and the second part on frozen ice packs at 4°C.

Upon receipt: (1) Store S6 at –20°C away from light; (2) Store S10 at room temperature away from light; (3) Store all other components at 4°C away from light. All components of the kit are stable for 6 months from the date of shipment, when stored properly.

Note: Check if wash buffer, S1, 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

- ☐ Orbital shaker
- ☐ Pipettes and pipette tips
- ☐ Microplate reader
- ☐ 1.5 ml microcentrifuge tubes
- ☐ Antibodies against proteins of interest

GENERAL PRODUCT INFORMATION

Usage Limitation: The *EpiQuik™ In Vivo* Universal Protein Sumoylation Assay Kit is for research use only and is not intended for diagnostic or therapeutic application.

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.

Intellectual Property: The *EpiQuik™ In Vivo* Universal Protein Sumoylation Assay 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.

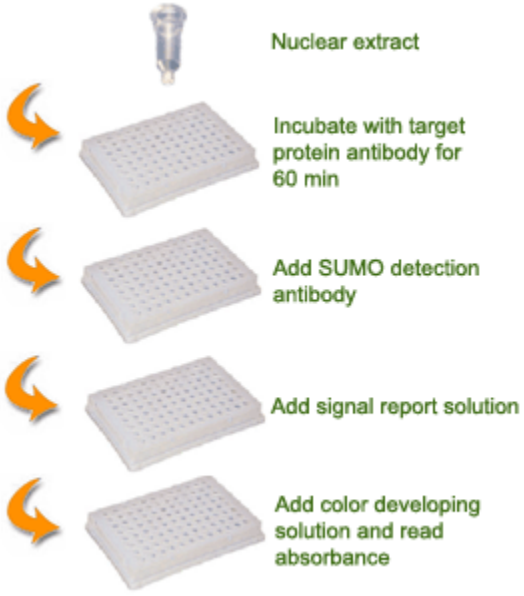
SUMO proteins are similar to ubiquitin. There are 3 confirmed SUMO isoforms in humans: SUMO-1, SUMO-2, and SUMO-3. SUMO-2/3 show a high degree of similarity to each other and are distinct from SUMO-1. Sumoylation is directed by an enzymatic cascade analogous to that involved in ubiquitination. Sumoylation 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 very few methods available for measuring *in vivo* protein sumoylation. The *EpiQuik™ In Vivo* Universal Protein Sumoylation Assay Kit addresses this problem, using a proprietary and unique procedure to measure *in vivo* protein sumoylation. The kit has the following features:

- Fast procedure, which can be finished within 5 hours.
- One-step colorimetric assay without the need for affinity chromatography and Western blotting.
- Flexible antibody choice allows the detection of sumoylation of multiple target proteins simultaneously.
- Included SUMO protein as the positive control 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* Universal Protein Sumoylation Assay 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.



Schematic Procedure for Using the *EpiQuik™ In Vivo* Universal Protein Sumoylation Assay Kit

ASSAY PROTOCOL

1. Prepare nuclear extracts from cells/tissues treated (e.g., Sumo-1 transfected) or untreated by using you own successful method. For your convenience and the best results, EpigenTek offers a nuclear extraction kit (Cat. No. OP-0002-1) optimized for use in *EpiQuik™* series. Nuclear extracts can be used immediately or stored at –80°C for future use.
2. 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 **S1** 10X Wash Buffer with distilled water (pH 7.2-7.5) at a 1:10 ratio (e.g., 1 ml of **S1** + 9 ml of distilled water).
3. Dilute your antibodies against the proteins of interest with **S2** to a final concentration of 2 µg/ml. The antibodies used should be IP-grade. For the sample wells, add 100 µl of the antibody solution. Dilute **S3** with **S2** also to a final concentration of 2 µg/ml. For the negative control wells, add 100 µl of the **Diluted S3**. Cover the wells with Parafilm M or foil and incubate at 4°C overnight or at 37°C for 2 hours.

4. Remove the solutions from each well. Add 150 µl of **S4** to the wells and incubate at room temperature for 45 minutes.
5. Aspirate and wash each well with 150 µl of **Diluted S1** three times.
6. Add 28 µl of **S5** to all wells, and 2 µl of nuclear extracts (5-10 µg) to the sample wells and the negative control wells. Mix, cover the wells, and incubate at room temperature for 60 minutes. For the positive control wells (using 8-Well Control Strips), dilute **S6** with **S5** to different concentrations (0.01-0.5 µg/µl, 4-6 points) and add 2 µl of **S6** at different concentrations instead of nuclear extract.
7. Aspirate and wash each well with 150 µl of the **Diluted S1** three times.
8. Prepare the **Detection Solution**. For each 1 ml to prepare, add 1 µl of **S7** and 0.5 µl of **S8** into each 10 µl of the **Diluted S1**; mix and incubate at room temperature for 10 minutes. Then add 20 µl of **S3**, mix and incubate at room temperature for 15 minutes. Lastly, add 960 µl of the **Diluted S1** and mix.
9. Add 50 µl of **Detection Solution** to each well and incubate at room temperature for 60 minutes on an orbital shaker (50-100 rpm).
10. Aspirate and wash each well with 150 µl of the **Diluted S1** six times.
11. Add 100 µl of **S9** into the wells and incubate at room temperature for 2-10 minutes away from light. Monitor the color development of the negative control and positive control wells. The color in the positive control wells should changed to brilliant-blue, while the color in the negative control wells does not change, or may only change to slight blue.
12. Add 50 µl of **S10** into the wells. Measure and read the absorbance on a microplate reader at 450 nm.

    Note: *If the strip well frame or strips do not fit the microplate reader, transfer the solution to a standard 96-well microplate and read absorbance on a microplate reader at 450 nm.*

13. 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–negative control OD) versus amount of **S6** added in the wells and determine the slope as delta OD/ng.

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

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

TROUBLESHOOTING

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 suitable 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

The negative control wells are contaminated with antibodies.	Ensure only negative control is added.
The wells are not sufficiently blocked with S4.	Increase blocking time to 60-90 minutes.
The well is not washed sufficiently.	Check if wash at each step is performed according to the protocol.
Overdevelopment.	Decrease development time in step 12.

RELATED PRODUCTS

P-8002 *EpiQuik™ In Vivo* HDAC1 Sumoylation Assay Kit