EpiQuik™ In Vivo HDAC1 Sumoylation Assay Kit

Base Catalog # P-8002

PLEASE READ THIS ENTIRE USER GUIDE BEFORE USE

The *EpiQuik™ In Vivo* HDAC1 Sumoylation Assay Kit is very suitable for measuring in vivo HDAC1 sumoylation from multiple mammalian cells/tissue types including human, mouse, and rat.

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KIT CONTENTS

Components	48 assays P-8002-48	96 assays P-8002-96
SS1 (10X Wash Buffer)	25 ml	50 ml
SS2 (Sumo Assay Buffer)	2 ml	4 ml
SS3 (Sumo Protein, 1 µg/µl)*	6 µl	12 µl
SS4 (Sumo Antibody, 1µg/µl)*	5 µl	10 µl
SS5 (Signal Report Solution)*	10 µl	20 µl
SS6 (Color Development Solution)	6 ml	12 ml
SS7 (Stop Solution)	3 ml	6 ml
Signal Enhancer*	120 µl	240 µl
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 SS3 and SS5 at –20°C away from light; (2) Store SS7 at room temperature away from light; (3) Store all other components at 4°C away from light. The kit is stable for up to 6 months from the shipment date, when stored properly.

Note: *Check if wash buffer, SS1, 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

GENERAL PRODUCT INFORMATION

Usage Limitation: The *EpiQuik™ In Vivo* HDAC1 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* HDAC1 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; where SUMO-2/3 show high 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 the target proteins correlates with inhibition of their functions.

HDAC1 is a class I histone acetylase that plays a critical role in transcriptional repression of gene expression and has been shown to be an integral components of multiprotein co-repressor complexes. It was recently observed that an important mechanism for governing HDAC1 activity is sumoylation, which acts by potentiating HDAC1 activity. Thus, the detection of *in vivo* HDAC1 sumoylation would provide useful information for understanding control mechanisms of the HDAC1 activity and HDAC1-participated transcription pathways.

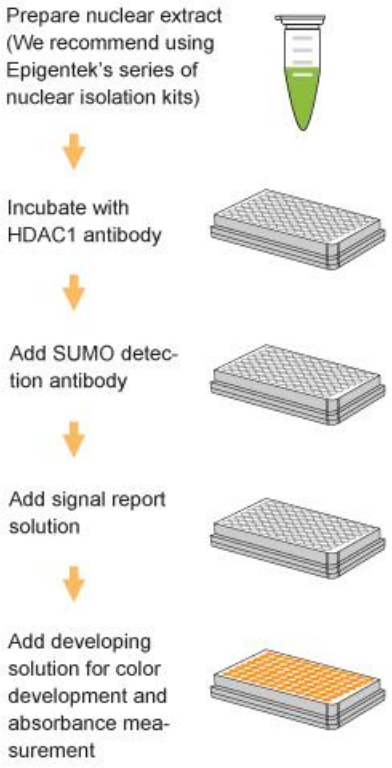
There are very few methods currently available for measuring *in vivo* HDAC1 sumoylation. The *EpiQuik™ In Vivo* HDAC1 Sumoylation Assay Kit addresses this problem and uses a proprietary and unique procedure to measure *in vivo* HDAC1 sumoylation. The kit has the following features:

- Fast procedure, which can be finished within 3 hours.
- One-step colorimetric assay without the use of affinity chromatography and Western blotting.
- Includes SUMO protein as the positive control allows HDAC1 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* HDAC1 Sumoylation Assay Kit is designed for measuring sumoylation of the targeted proteins. Sumoylation of HDAC1 is indicated by SUMO conjugated to this protein. In an assay with this kit, the antibody specific for the HDAC1 is stably coated on the strip wells. HDAC1

contained in the nuclear extracts is captured by the antibody. Sumoylation of HDAC1 can then be detected by recognition of SUMO conjugated to HDAC1 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* HDAC1 Sumoylation Assay Kit

PROTOCOL

1. Prepare nuclear extracts from cells/tissues treated (e.g., Sumo activation or inhibition) 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 **SS1** 10X Wash Buffer with distilled water (pH 7.2-7.5) at a 1:10 ratio (e.g., 1 ml of **SS1** + 9 ml of distilled water).

3. Add 28 µl of **SS2** and 2 µl of nuclear extracts (5-10 µg) to the sample wells. Mix, cover the wells, and incubate at room temperature for 60 minutes. For the blank wells, add 30 µl of **SS2**. For the

positive control wells, dilute **SS3** with **SS2** at different concentrations (0.01-0.25 µg/µl) and add 2 µl of **SS3** at these varying concentrations instead of nuclear extract.

4. Aspirate and wash each well with 150 µl of the **Diluted SS1** three times.

5. Prepare the **Detection Solution**. For each 1 ml to prepare, add 2 µl of **SS4** and 0.5 µl of **SS5** into each 10 µl of the **Diluted SS1**; mix and incubate at room temperature for 10 minutes. Then add 20 µl of **Signal Enhancer**, mix and incubate at room temperature for 15 minutes. Lastly, add 970 µl of the **Diluted SS1** and mix.

6. Add 50 µl of **Detection Solution** to each well and incubate at room temperature for 60 minutes on an orbital shaker (50-100 rpm).

7. Aspirate and wash each well with 150 µl of the **Diluted SS1** six times.

8. Add 100 µl of **SS6** into the wells and incubate at room temperature for 2 to 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 change to brilliant-blue, while the color in the blank wells have not changed or may only change to a slight blue tinge.

9. Add 50 µl of **SS7** into the wells to stop color development. Measure and read absorbance on a microplate reader at 450 nm.

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

10. Calculate sumoylation of HDAC1.

    For simple calculation:

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

    For accurate calculation:

    (1) Plot Delta OD values (positive control OD–negative control OD) versus amount of **SS3** added in the wells and determine the slope as delta OD/ng.
    (2) Calculate intensity of the conjugated SUMO using the following formula:

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

TROUBLESHOOTING

No Signal for the Sample

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 incorrectly stored.	Ensure the nuclear extracts are stored at –80°C.
Reagents are added incorrectly	Check if reagents are added in proper order and if any steps of the procedure may have been 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 Blank

The blank wells are contaminated with positive control protein.	Ensure no positive control protein is added.
The well is not washed sufficiently.	Check if wash at each step is performed according to protocol.
Overdevelopment.	Decrease development time in step 12.

RELATED PRODUCTS

P-8001 EpiQuik™ In Vivo Universal Protein Sumoylation Assay Kit