ab115132 – HDAC1 Sumoylation Assay Kit

Instructions for Use

For the measurement of sumoylation of targeted proteins

This product is for research use only and is not intended for diagnostic use.

Version 1 Last Updated 3 September 2014

Table of Contents

1. BACKGROUND

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.

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. ab115132 Kit addresses this problem and uses a proprietary and unique procedure to measure in vivo HDAC1 sumoylation.

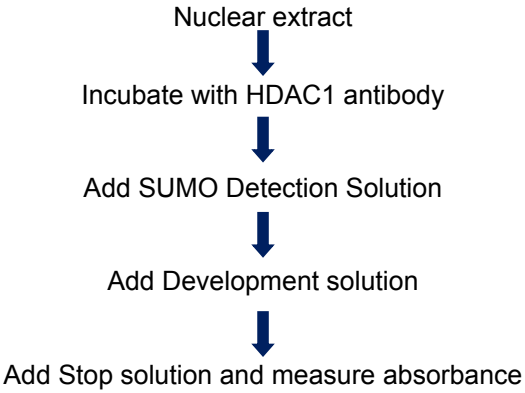
This kit has the following features:

- 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 allowing HDAC1 sumoylation to be quantified

- Strip microplate format makes the assay flexible: manual or high throughput
- Reliable and consistent assay conditions

Abcam's 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.

2. ASSAY SUMMARY



3. PRECAUTIONS

Please read these instructions carefully prior to beginning the assay.

All kit components have been formulated and quality control tested to function successfully as a kit. Modifications to the kit components or procedures may result in loss of performance.

4. STORAGE AND STABILITY

Store kit as given in the table and away from light upon receipt.

Observe the storage conditions for individual prepared components in sections 9 & 10.

For maximum recovery of the products, centrifuge the original vial prior to opening the cap.

Check if the 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.

5. MATERIALS SUPPLIED

Item	48 Tests	96 Tests	Storage Condition (Before Preparation)
10X Wash Buffer	25 mL	50 mL	4°C
Sumo Assay Buffer	2 mL	4 mL	4°C
Sumo Protein, 1 µg/µL	6 µL	12 µL	-20°C
Sumo Antibody, 1 µg/µL	5 µL	10 µL	4°C
Signal Report Solution	10 µL	20 µL	-20°C
Color Development Solution	6 mL	12 mL	4°C
Stop Solution	3 mL	6 mL	4°C
Signal Enhancer	120 µL	240 µL	4°C
8-Well Assay Strip (with Frame)	6	12	4°C
8-Well Control Strips	2	3	4°C

6. MATERIALS REQUIRED, NOT SUPPLIED

These materials are not included in the kit, but will be required to successfully utilize this assay:

- Orbital shaker
- Pipettes and pipette tips
- Microplate reader
- 1.5 mL microcentrifuge tubes

7. LIMITATIONS

- Assay kit intended for research use only. Not for use in diagnostic procedures
- Do not use kit or components if it has exceeded the expiration date on the kit labels
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted
- Any variation in operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding

8. TECHNICAL HINTS

- Avoid foaming or bubbles when mixing or reconstituting components
- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions
- Ensure plates are properly sealed or covered during incubation steps
- Complete removal of all solutions and buffers during wash steps
- **This kit is sold based on number of tests. A 'test' simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions**

9. REAGENT PREPARATION

10.1 1X Wash Buffer

Dilute 10X Wash Buffer with distilled water (pH 7.2-7.5) at a 1:10 ratio (1 mL of 10X Wash Buffer + 9 mL of water).This diluted 1X Wash Buffer can now be stored at 4°C for up to six months.

10.2 Detection Solution

For each 1 mL to prepare, add 2 µL of the Sumo Antibody and 0.5 µL of the Signal Report Solution into each 10 µL of 1X Wash Buffer; mix and incubate at room temperature for 10 minutes. Then add 20 µL of the Signal Enhancer, mix and incubate at room temperature for 15 minutes. Lastly, add 970 µL of 1X Wash Buffer and mix.

10. SAMPLE PREPARATION

Prepare nuclear extracts from cells/tissues treated (e.g. Sumo activation or inhibition) or untreated by using your own successful method. For your convenience and the best results, Abcam offers a Nuclear Extraction Kit (ab113474).

Nuclear extracts can be used immediately or stored at -80°C for future use.

11. ASSAY PROCEDURE

11.1 Predetermine the number of strip wells required. Remove un-needed strip wells from the plate frame and place them back in the bag (seal the bag tightly and store at 4°C).

11.2 Add 28 µL of Sumo Assay Buffer 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 blank wells, add 30 µL of Sumo Assay Buffer. For the positive control wells dilute Sumo Protein with Sumo Assay Buffer to different concentrations (0.01-0.25 µg/µL) and add 2 µL of Sumo Protein at different concentrations instead of nuclear extract.

11.3 Aspirate and wash each well with 150 µL of 1X Wash Buffer three times.

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

11.5 Aspirate and wash each well with 150 µL of 1X Wash Buffer six times.

11.6 Add 100 µL of the Color Development Solution 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 change to brilliant-blue, while the color in the blank wells have not changed or may only change to slight blue.

11.7 Add 50 µL of Stop Solution into the wells. Measure and read 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.*

11.8 Calculate % sumoylation of HDAC1 using the formulae provided in Section 12 – Data Analysis.

12. ANALYSIS

Calculate the % sumoylation of HDAC1 using the following formula:

$$\% \text{ Sumoylation} = \frac{\text{Treated Sample OD} - \text{Negative Control OD}}{\text{Untreated Sample OD} - \text{Negative Control OD}} \times 100\%$$

For an accurate quantification, plot delta OD values (positive control OD-negative control OD) versus amount of Sumo Protein added in the wells and determine the slope as delta OD/ng.

Calculate intensity of the conjugated SUMO using the following formula:

$$\text{Sumoylation intensity (ng/mg protein)} = \frac{\text{Sample OD} - \text{Negative Control OD}}{\text{Protein amount added (µg)* x Slope}} \times 1000$$

*Nuclear extract amount added into the sample well at step 11.2.

13. TROUBLESHOOTING

Problem	Cause	Solution
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 the procedure and reagents are correct for the nuclear protein extraction
	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 are omitted by mistake
	Incubation time and temperature is incorrect	Ensure the incubation time and temperature described in the protocol is 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 the protocol
	Overdevelopment	Decrease development time in step 11.6.

14. NOTES

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