ab115131 – Global Protein 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 3b Last Updated 15 January 2020

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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. 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 currently available for measuring in vivo protein sumoylation. ab115131 Kit addresses this problem and uses a proprietary and unique procedure to measure in vivo protein sumoylation..

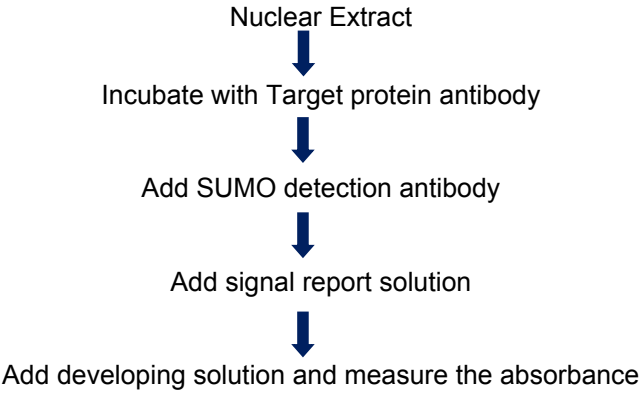
This kit has the following advantages and features:

- 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
- Reliable and consistent assay conditions

The Global 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.

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 upon receipt away from light.

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 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	2 x 25 mL	4°C
Binding Buffer	15 mL	30 mL	4°C
Negative Control, 1 µg/µL	120 µL	240 µL	4°C
Blocking Buffer	10 mL	20 mL	4°C
Sumo Assay Buffer	2 mL	4 mL	4°C
Sumo Protein, 1 µg/µL	10 µL	20 µL	-20°C
Sumo Antibody, 1 µg/µL	5 µL	10 µL	4°C
Signal Report Solution	5 µL	10 µL	4°C
Color Development Solution	6 mL	12 mL	4°C
Stop Solution	3 mL	6 mL	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
- Antibodies against proteins of interest

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

1X Wash Buffer

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

10. SAMPLE PREPARATION

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, Abcam offers an Nuclear Extraction Kit (ab113474) optimized for use with this kit.

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

11. ASSAY PROCEDURE

11.1 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).

11.2 Dilute your antibodies against the proteins of interest with Binding Buffer 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 Negative Control with Binding Buffer also to a final concentration of 2 µg/mL. For the negative control wells, add 100 µL of the Diluted Negative Control. Cover the wells with Parafilm M or foil and incubate at 4°C overnight or at 37°C for 2 hours.

11.3 Remove the solutions from each well. Add 150 µL of Blocking Buffer to the wells and incubate at room temperature for 45 minutes.

11.4 Aspirate and wash each well with 150 µl of Diluted Wash Buffer three times.

11.5 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 positive control wells (using 8-Well Control Strips), dilute Sumo Protein with Sumo Assay Buffer to different concentrations (0.01-0.5 µg/ µL, 4-6 points) and add 2 µL of Sumo Protein at different concentrations instead of nuclear extract.

11.6 Aspirate and wash each well with 150 µL of the Diluted Wash Buffer three times.

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

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

11.9 Aspirate and wash each well with 150 µL of the Diluted Wash Buffer six times.

11.10 Add 100 µL of 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 negative control wells does not change, or may only change to slight blue.

11.11 Add 50 µL of Stop Solution 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.*

12. ANALYSIS

12.1 **Simple Calculation**

$$\% \text{ Sumoylation} = \frac{\text{Treated sample OD} - \text{negative control OD}}{\text{Untreated sample OD} - \text{negative control OD}} \times 100\%$$

12.2 **Accurate Calculation**

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:

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

13. TROUBLESHOOTING

Problem	Cause	Solution
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 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
No Signal for the Sample	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 Blocking Buffer.	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 11.10.

14. NOTES

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