# Human SUMO1 ELISA Kit
# Part hSUMO1ELISA-biotin

FIVEphoton Biochemicals

**For research use only.**
**Not for diagnostics.**

Part No. hSUMO1ELISA-biotin

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**This protocol is provided as a guide. Use the protocol shipped with the kit for your experiment.**

**Store:** 2-8$^{o}$C. Expiration: 12 months after arrival.
**Safety:** Stop solution contains acid. Avoid contact and inhalation. Wear eye protection.

# SUMO1 ELISA Kit

**Part No.** hSUMO1ELISA-biotin

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Safety: Stop solution contains acid. Avoid eye and skin contact

Standard Peptide: 48 ng/ml

Assay Range: 150 pg/ml - 40 ng/ml

**Overview:** Please read this protocol completely prior to using the product. The kit measures Human SUMO1 and proteins conjugated with it in sandwich ELISA format. The ELISA plate is provided pre-coated with an anti-SUMO1 monoclonal antibody. The experimenter mixes the samples with a provided polyclonal anti-SUMO1 antibody labeled with biotin and with the provided streptavidin-HRP solution *prior* to the addition of the mixture to the ELISA plate. The sample-antibody-biotin-streptavidin-HRP mixture is added to the ELISA plate, which is incubated for 60 min at 37$^{o}$C. Unbound biotin-linked antibody is washed away and color reagents are added that change the color of the solution to blue. The blue solution turns yellow after addition of the stop solution. The absorbance of the resulting yellow solution at 450nm corresponds to the concentration of total Human SUMO1 in the samples.

**Sumolyated Protein Isolation**

SUMO1 is conjugated to the cytosolic domains of proteins as a post-translational modification with a mechanism similar to ubiquitin attachment. Proteins modified with SUMO1 can translocate and reside in the nucleus and perform gene regulatory roles. Therefore, the primary mechanism to isolate sumolyated proteins involves cell lysis to isolate a total sumolyated protein pool from all cellular compartments, or nuclear-cytoplasmic fractionation to partition cytosolic and nuclear sumolyated proteins. The Fivephoton Biochemicals ELISA Lysis Buffer (Part ELSP-1), or similar, can be used to isolate total cellular sumolyated protein; the Fivephoton Biochemicals Nuclear Protein Isolation Kit (Part NPI-1), or similar, can be employed to partition nuclear from cytoplasmic sumolyated protein for ELISA measurements.

**Table 1. Materials supplied with the kit. Store all materials at 4$^{o}$C**

| | | | | | |
|---|---|---|---|---|---|
| 1 | Standard peptide: 48 ng/ml | 0.5ml | 7 | Chromogen Solution A | 6 ml |
| 2 | Standard diluents (1% BSA in PBS pH 7.4 | 3 ml | 8 | Chromogen Solution B | 6 ml |
| 3 | Anti-SUMO1 Coated ELISA plate (96T) | 12 well×8 strips | 9 | Stop Solution | 6 ml |
| 4 | Streptavidin-HRP | 6 ml | 10 | Instruction Manual | 1 |
| 5 | 30×wash solution | 20 ml | 11 | Closure Membrane | 2 |
| 6 | Anti SUMO1 antibody labeled with biotin (detection antibody) | 1ml | 12 | Sealed bags | 1 |
| | | | | | |

**Materials required but not supplied**

1. Sample dilution buffer (1% BSA in PBS pH 7.4)

2. 37°C incubator
3. Standard absorbance plate reader
4. Precision pipettes and disposable pipette tips
5. De-ionized water
6. Disposable tubes for sample dilution
7. Absorbent paper

**Important notes and preparation for the assay**

1. Make a solution that is 1% BSA in PBS pH 7.4 for dilution of samples (i.e." sample dilution solution").
2. Equilibrate the ELISA plate strips to room temperature for at least 30 minutes. The plate strips should be kept in a zip lock bag to prevent drying of the wells. Store unused sections of the ELISA plate in a sealed bag at 4°C.
3. Use new pipettes tips at each step to prevent contamination.
4. Do not use reagents from other ELISA kits..
5. Substrate B is light sensitive. Limit light exposure.
6. **Manual Wash Method:** Gently aspirate off the liquid in each well. Turn over the plate and pat-dry on absorbent paper. Add 100µl wash solution and repeat 5 times. Therefore 600ul wash solution is needed per well. An automatic washer can also be employed to wash the ELISA wells.

## Assay procedures

Blanks, standard and sample preparation Prepare the blanks, standards and samples in a separate 96-well dish and transfer simultaneously to the ELISA plate. Note that the sample – detection antibody – HRP complex are prepared prior to addition of the samples to wells. Do not prepare samples in the ELISA plate.

**Assay procedure**

1. Standard dilutions. Use Table 2 as a guide for dilutions of standards in the provided standard diluent solution. Make dilutions in separate tubes or multi-well dishes, not in the ELISA dish. Use 2 replicates for the blank, standard and sample wells.

**Table 2. Standard Dilutions**

| Standard Concentration | Standard Number | Dilution Instructions |
|---|---|---|
| 24 ng/ml | 7 | 120µl original standard + 120µl standard diluents solution, mix |
| 12 ng/ml | 6 | 120µl standard No. 7 + 120µl standard diluents solution, mix |
| 6 ng/ml | 5 | 120µl standard No. 6 + 120µl standard diluents solution, mix |
| 3 ng/ml | 4 | 120µl standard No. 5 + 120µl standard diluents solution, mix |
| 1.5 ng/ml | 3 | 120µl standard No. 4 + 120µl standard diluents solution, mix |
| 0..75 ng/ml | 2 | 120µl standard No .3 + 120µl standard diluents solution, mix |
| 0.375 ng/ml | 1 | 120µl standard No. 2 + 120µl standard diluents solution, mix |

2. Blank, standard and sample preparation prior to addition to the ELISA wells:

a) Blank wells: Set aside 2 blank wells. Only add chromogen solutions A and B and then stop solution later during the procedural steps while developing the ELISA.

b) Standard solution preparation: Add 50µl standard (that was previously diluted) and 50µl streptavidin-HRP. (Detection antibody labeled with biotin has been added in advance to the standards, therefore detection antibody is not added to prepare the standards solutions).

c) Sample wells: Dilute 10ul sample in 30µl sample diluent solution (1% BSA in PBS pH 7.4 – sample diluent is made by the experimenter), then add 10µl SUMO1 detection-biotin antibody and 50µl streptavidin-HRP. Mix gently; do not vortex. Note that the sample was diluted 1:4 in this step: account this dilution when calculating the concentration of SUMO1 in the samiple.

d) Transfer the standard and sample solutions simultaneously to the ELISA dish. Seal the wells with a ziplock bag and rotate the plate gently for 60 minutes at 37$^{o}$C.

3. Preparation of wash solution: Dilute the 30X wash solution to 1X with $dH_20$. Prepare 600µl 1X wash solution per well.

4. Wash: Carefully remove the seal plate membrane or zip lock bag that protects the wells from drying as not to cross-contaminate liquid. Aspirate off the liquid. Fill each well with 100µl 1X wash solution. Aspirate the liquid after 30 seconds. Then repeat the wash procedure five times. Quickly blot dry the plate.

5. Color development: First add 50µl chromogen solution A to each well and then add 50µl chromogen solution B to each well. Shake gently to mix solutions A and B. Incubate the plate protected from light for 10 minutes at 37$^{o}$C.

6. Stop solution: Add 50ìl Stop Solution to each well to stop the reaction (the blue color changes into yellow).

7. Read samples at 450nm within 10 min of adding the stop solution: Set the blank well as zero, measure the absorbance (OD) of each well at 450nm.

**Data Analysis**

1. Compile a standard curve using the blank standard solutions and the corresponding OD values. You may wish to calculate a linear regression equation from the standard curve to determine the concentration of your samples. Take into account dilution factors in your calculation of SUMO1 concentration. Alternative methods of data analysis to calculate concentrations of your samples can also be employed.

**Flow chart of the procedures**

Separately prepare standards, blank and samples with detection antibody and HRP-streptavidin as required

Incubate with the ELISA plate for 1 hr at 37$^{o}$C.

Wash the plate five times.

Add Chromogen solutions A and B. Incubate for 10 min at 37$^{o}$C for color development.

Add stop solution

**Measure OD value at 450nm within 10 min**