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ab178012 – Human SOD2 SimpleStep ELISA® Kit (Superoxide Dismutase 2)

For the quantitative measurement of SOD2 (Superoxide Dismutase 2) in human cell and tissue extracts.

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

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1. Overview

SOD2 (Superoxide Dismutase 2) *in vitro* SimpleStep ELISA® (Enzyme-Linked Immunosorbent Assay) kit is designed for the quantitative measurement of SOD2 protein in human cell and tissue extracts.

The SimpleStep ELISA® employs an affinity tag labeled capture antibody and a reporter conjugated detector antibody which immunocapture the sample analyte in solution. This entire complex (capture antibody/analyte/detector antibody) is in turn immobilized via immunoaffinity of an anti-tag antibody coating the well. To perform the assay, samples or standards are added to the wells, followed by the antibody mix. After incubation, the wells are washed to remove unbound material. TMB Development Solution is added and during incubation is catalyzed by HRP, generating blue coloration. This reaction is then stopped by addition of Stop Solution completing any color change from blue to yellow. Signal is generated proportionally to the amount of bound analyte and the intensity is measured at 450 nm. Optionally, instead of the endpoint reading, development of TMB can be recorded kinetically at 600 nm.

The principle cellular anti-oxidant enzymes are the superoxide dismutase family (SOD, E.C. 1.15.1.1). These enzymes dismutate superoxide into hydrogen peroxide which is further detoxified by other cellular defenses such as glutathione peroxidase and catalase. Superoxide and its products have been implicated in a wide range of diseases including cancer, inflammation, neurodegenerative diseases, diabetes and aging. The SOD family has 3 members, two of which are Cu-Zn type – the extracellular SOD3 and the cytoplasmic SOD1. The other member is the mitochondrial Mn (manganese) type SOD2. The mitochondrial Mn-SOD2 is a homotetramer of subunit mass 23 kDa in the mitochondrial matrix. SOD2 has been shown to be essential since knockout mice die shortly after birth. SOD2 levels may be downregulated in tumor cells and studies show that over expression of SOD2 in tumor cells may suppress cell division and cancer growth (Oberley, Biomedecine & Pharmacotherapy, 2005, 59, p143-8).

2. Protocol Summary

Remove appropriate number of antibody coated well strips. Prepare all reagents, samples, and standards as instructed

Add standard or sample to appropriate wells.

Incubate at room temperature.

Add Antibody Cocktail to all wells. Incubate at room temperature.

Aspirate and wash each well. Add TMB Development Solution to each well and incubate.

Add Stop Solution at a defined endpoint.

Alternatively, record color development kinetically after TMB substrate addition.

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.
- We understand that, occasionally, experimental protocols might need to be modified to meet unique experimental circumstances. However, we cannot guarantee the performance of the product outside the conditions detailed in this protocol booklet.
- Reagents should be treated as possible mutagens and should be handle with care and disposed of properly. Please review the Safety Datasheet (SDS) provided with the product for information on the specific components.
- Observe good laboratory practices. Gloves, lab coat, and protective eyewear should always be worn. Never pipet by mouth. Do not eat, drink or smoke in the laboratory areas.
- All biological materials should be treated as potentially hazardous and handled as such. They should be disposed of in accordance with established safety procedures.

4. Storage and Stability

Store kit at +4°C immediately upon receipt. Kit has a storage time of 1 year from receipt, providing components have not been reconstituted.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in the Materials Supplied section.

5. Limitations

- Assay kit intended for research use only. Not for use in diagnostic procedures.
- 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.

6. Materials Supplied

Item	Amount	Storage Condition
10X SOD2 Capture Antibody	600 µL	+2-8°C
10X SOD2 Detector Antibody	600 µL	+2-8°C
SOD2 Human Lyophilized Recombinant Protein	2 Vials	+2-8°C
Antibody Diluent 5B	6 mL	+2-8°C
10X Wash Buffer PT	20 mL	+2-8°C
5X Cell Extraction Buffer PTR	10 mL	+2-8°C
50X Cell Extraction Enhancer Solution	1 mL	+2-8°C
TMB Development Solution	12 mL	+2-8°C
Stop Solution	12 mL	+2-8°C
Sample Diluent NS*	12 mL	+2-8°C
Pre-Coated 96 Well Microplate (12 x 8 well strips)	96 Wells	+2-8°C
Plate Seal	1	+2-8°C

*Sample Diluent NS is provided but not needed for this product.

7. Materials Required, Not Supplied

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

- Microplate reader capable of measuring absorbance at 450 or 600 nm.
- Method for determining protein concentration (BCA assay recommended).
- Deionized water.
- Multi- and single-channel pipettes.
- Tubes for standard dilution.
- Plate shaker for all incubation steps.
- Optional: Phenylmethylsulfonyl Fluoride (PMSF) (or other protease inhibitors).

8. Technical Hints

- Samples generating values higher than the highest standard should be further diluted in the appropriate sample dilution buffers.
- 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 is necessary to minimize background.
- As a guide, typical ranges of sample concentration for commonly used sample types are shown below in Sample Preparation (section 11).
- All samples should be mixed thoroughly and gently.
- Avoid multiple freeze/thaw of samples.
- Incubate ELISA plates on a plate shaker during all incubation steps.
- When generating positive control samples, it is advisable to change pipette tips after each step.

- The provided Cell Extraction Enhancer Solution 50X may precipitate when stored at + 4ºC. To dissolve, warm briefly at + 37ºC and mix gently. The Cell Extraction Enhancer Solution 50X can be stored at room temperature to avoid precipitation.
- **To avoid high background always add samples or standards to the well before the addition of the antibody cocktail.**
- **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

- Equilibrate all reagents to room temperature (18-25°C) prior to use. The kit contains enough reagents for 96 wells. The sample volumes below are sufficient for 48 wells (6 x 8-well strips); adjust volumes as needed for the number of strips in your experiment.
- Prepare only as much reagent as is needed on the day of the experiment. Capture and Detector Antibodies have only been tested for stability in the provided 10X formulations.

9.1 1X Cell Extraction Buffer PTR

Prepare 1X Cell Extraction Buffer PTR by diluting 5X Cell Extraction Buffer PTR and 50X Cell Extraction Enhancer Solution to 1X with deionized water. To make 10 mL 1X Cell Extraction Buffer PTR combine 7.8 mL deionized water, 2 mL 5X Cell Extraction Buffer PTR and 200 µL 50X Cell Extraction Enhancer Solution Mix thoroughly and gently. If required protease inhibitors can be added.
Alternative – Enhancer may be added to 1X Cell Extraction Buffer PTR after extraction of cells or tissue. Refer to note in Section 19.

9.2 1X Wash Buffer

Prepare 1X Wash Buffer PT by diluting 10X Wash Buffer PT with deionized water. To make 50 mL 1X Wash Buffer PT combine 5 mL 10X Wash Buffer PT with 45 mL deionized water. Mix thoroughly and gently.

9.3 Antibody Cocktail

Prepare Antibody Cocktail by diluting the capture and detector antibodies in Antibody Diluent 5B. To make 3 mL of the Antibody Cocktail combine 300 µL 10X Capture Antibody and 300 µL 10X Detector Antibody with 2.4 mL Antibody Diluent 5B. Mix thoroughly and gently.

10. Standard Preparation

- Always prepare a fresh set of standards for every use.
- Discard working standard dilutions after use as they do not store well.
- The following section describes the preparation of a standard curve for duplicate measurements (recommended).

10.1 IMPORTANT: If the protein standard vial has a volume identified on the label, reconstitute the SOD2 standard by adding that volume of 1X Cell Extraction Buffer PTR indicated on the label. Alternatively, if the vial has a mass identified, reconstitute the SOD2 standard by adding 200 µL 1X Cell Extraction Buffer PTR. Hold at room temperature for 10 minutes and mix gently. This is the 100 ng/mL Stock Standard Solution.

10.2 Label eight tubes, Standards 1– 8.

10.3 Add 150 µL 1X Cell Extraction Buffer PTR into each tube.

10.4 Add 150 µL of stock standard into tube 1.

10.5 Prepare tube two by transferring 150 µL from tube 1 into tube 2. Repeat for tube #3 to #7.

10.6 Use the Stock Standard to prepare the following dilution series. Standard #8 contains no protein and is the Blank control:

Standard #	Volume to dilute (µL)	Volume Diluent N (µL)	(ng/mL)
1	Step 10.4		50
2	150 µL Standard #1	150	25
3	150 µL Standard #2	150	12.5
4	150 µL Standard #3	150	6.25
5	150 µL Standard #4	150	3.12
6	150 µL Standard #5	150	1.56
7	150 µL Standard #6	150	0.78
8 (Blank)	N/A	150	0

11. Sample Preparation

TYPICAL SAMPLE DYNAMIC RANGE	
Sample Type	**Range**
HepG2 cell extract	0.06 – 4 µg/mL

11.1 Preparation of extracts from cell pellets

- Collect non-adherent cells by centrifugation or scrape to collect adherent cells from the culture flask. Typical centrifugation conditions for cells are 500 x g for 5 minutes at 4°C.
- Rinse cells twice with PBS.
- Solubilize pellet at 2x107 cell/mL in chilled 1X Cell Extraction Buffer PTR.
- Incubate on ice for 20 minutes.
- Centrifuge at 18,000 x g for 20 minutes at 4°C.
- Transfer the supernatants into clean tubes and discard the pellets.
- Assay samples immediately or aliquot and store at 80°C. The sample protein concentration in the extract may be quantified using a protein assay.
- Dilute samples to desired concentration in 1X Cell Extraction Buffer PTR.

11.2 Preparation of extracts from adherent cells by direct lysis (alternative protocol)

- Remove growth media and rinse adherent cells 2 times in PBS.
- Solubilize the cells by addition of chilled 1X Cell Extraction Buffer PTR directly to the plate (use 750 µL - 1.5 mL 1X Cell Extraction Buffer PTR per confluent 15 cm diameter plate).
- Scrape the cells into a microfuge tube and incubate the lysate on ice for 15 minutes.
- Centrifuge at 18,000 x g for 20 minutes at 4°C.
- Transfer the supernatants into clean tubes and discard the pellets.
- Assay samples immediately or aliquot and store at 80°C. The sample protein concentration in the extract may be quantified using a protein assay.

- Dilute samples to desired concentration in 1X Cell Extraction Buffer PTR.

11.3 Preparation of extracts from tissue homogenates

- Tissue lysates are typically prepared by homogenization of tissue that is first minced and thoroughly rinsed in PBS to remove blood (dounce homogenizer recommended).
- Homogenize 100 to 200 mg of wet tissue in 500 µL 1 mL of chilled 1X Cell Extraction Buffer PTR. For lower amounts of tissue adjust volumes accordingly.
- Incubate on ice for 20 minutes.
- Centrifuge at 18,000 x g for 20 minutes at 4°C.
- Transfer the supernatants into clean tubes and discard the pellets.
- Assay samples immediately or aliquot and store at 80°C. The sample protein concentration in the extract may be quantified using Dilute samples to desired concentration in 1X Cell Extraction Buffer PTR.

Guidelines for Dilutions of 100-fold or Greater *(for reference only; please follow the insert for specific dilution suggested)*	
100x	**10000x**
4 µl sample + 396 µl buffer (100X) = 100-fold dilution *Assuming the needed volume is less than or equal to 400 µl*	A) 4 µl sample + 396 µl buffer (100X) B) 4 µl of A + 396 µl buffer (100X) = 10000-fold dilution *Assuming the needed volume is less than or equal to 400 µl*
1000x	**100000x**
A) 4 µl sample + 396 µl buffer (100X) B) 24 µl of A + 216 µl buffer (10X) = 1000-fold dilution *Assuming the needed volume is less than or equal to 240 µl*	A) 4 µl sample + 396 µl buffer (100X) B) 4 µl of A + 396 µl buffer (100X) C) 24 µl of A + 216 µl buffer (10X) = 100000-fold dilution *Assuming the needed volume is less than or equal to 240 µl*

Refer to Dilution Guidelines for further instruction.

12. Plate Preparation

- The 96 well plate strips included with this kit are supplied ready to use. It is not necessary to rinse the plate prior to adding reagents.
- Unused plate strips should be immediately returned to the foil pouch containing the desiccant pack, resealed and stored at 4°C.
- For each assay performed, a minimum of two wells must be used as the zero control.
- For statistical reasons, we recommend each sample should be assayed with a minimum of two replicates (duplicates).
- Differences in well absorbance or "edge effects" have not been observed with this assay.

13. Assay Procedure

- Equilibrate all materials and prepared reagents to room temperature prior to use.
- We recommend that you assay all standards, controls and samples in duplicate.

13.1 Prepare all reagents, working standards, and samples as directed in the previous sections.

13.2 Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, reseal and return to 4ºC storage.

13.3 Add 50 µL of all sample or standard to appropriate wells.

13.4 Add 50 µL of the Antibody Cocktail to each well.

13.5 Seal the plate and incubate for 1 hour at room temperature on a plate shaker set to 400 rpm.

13.6 Wash each well with 3 x 350 µL 1X Wash Buffer PT. Wash by aspirating or decanting from wells then dispensing 350 µL 1X Wash Buffer PT into each well. Complete removal of liquid at each step is essential for good performance. After the last wash invert the plate and blot it against clean paper towels to remove excess liquid.

13.7 Add 100 µL of TMB Development Solution to each well and incubate for 10 minutes in the dark on a plate shaker set to 400 rpm.

Given variability in laboratory environmental conditions, optimal incubation time may vary between 5 and 20 minutes.
Note: The addition of Stop Solution will change the color from blue to yellow and enhance the signal intensity about 3X. To avoid signal saturation, proceed to the next step before the high concentration of the standard reaches a blue color of O.D.600 equal to 1.0.

13.8 Add 100 µL of Stop Solution to each well. Shake plate on a plate shaker for 1 minute to mix. Record the OD at 450 nm. This is an endpoint reading.

13.9 Alternative to 13.7 – 13.8: Instead of the endpoint reading at 450 nm, record the development of TMB Substrate kinetically. Immediately after addition of TMB Development Solution begin recording the blue color development with elapsed time in the microplate reader prepared with the following settings:

Mode	Kinetic
Wavelength:	600 nm
Time:	up to 20 min
Interval:	20 sec - 1 min
Shaking:	Shake between readings

Δ **Note**: that an endpoint reading can also be recorded at the completion of the kinetic read by adding 100 µL Stop Solution to each well and recording the OD at 450 nm.

13.10 Analyze the data as described below.

14. Calculations

14.1 Calculate the average absorbance value for the blank control (zero) standards. Subtract the average blank control standard absorbance value from all other absorbance values.

14.2 **Create a standard curve** by plotting the average blank control subtracted absorbance value for each standard concentration (y-axis) against the target protein concentration (x-axis) of the standard. Use graphing software to draw the best smooth curve through these points to construct the standard curve.

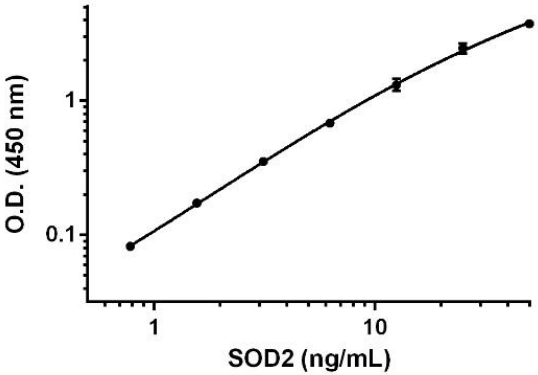
Δ **Note:** Most microplate reader software or graphing software will plot these values and fit a curve to the data. A four parameter curve fit (4PL) is often the best choice; however, other algorithms (e.g. linear, semi-log, log/log, 4 parameter logistic) can also be tested to determine if it provides a better curve fit to the standard values.

14.3 Determine the concentration of the target protein in the sample by interpolating the blank control subtracted **absorbance values against the standard curve**. Multiply the resulting value by the appropriate sample dilution factor, if used, to obtain the concentration of target protein in the sample.

14.4 Samples generating absorbance values greater than that of the highest standard should be further diluted and reanalyzed. Similarly, samples which measure at an absorbance values less than that of the lowest standard should be retested in a less dilute form.

15. Typical Data

Typical standard curve – data provided **for demonstration purposes only**. A new standard curve must be generated for each assay performed.



Standard Curve Measurements			
Conc. (ng/mL)	O.D. 450 nm		Mean O.D.
	1	2	
0.00	0.091	0.088	0.090
0.78	0.176	0.168	0.172
1.56	0.260	0.265	0.263
3.12	0.428	0.456	0.443
6.25	0.779	0.766	0.773
12.5	1.499	1.313	1.407
25	2.706	2.409	2.558
50	3.956	3.739	3.848

Figure 1. Example of SOD2 standard curve. The standard curve was prepared as described in Section 10. Raw data values are shown in the table. Background-subtracted data values (mean +/- SD) are graphed.

16. Typical Sample Values

SENSITIVITY –

The calculated minimal detectable (MDD) dose is 0.22 ng/mL. The MDD was determined by calculating the mean of zero standard replicates (n=30) and adding 2 standard deviations then extrapolating the corresponding concentrations.

PRECISION –

Mean coefficient of variations of interpolated values from 3 concentrations of HepG2 extracts within the working range of the assay.

	Intra-assay Precision	Inter-Assay Precision
n=	5	3
CV (%)	3.8	4.2

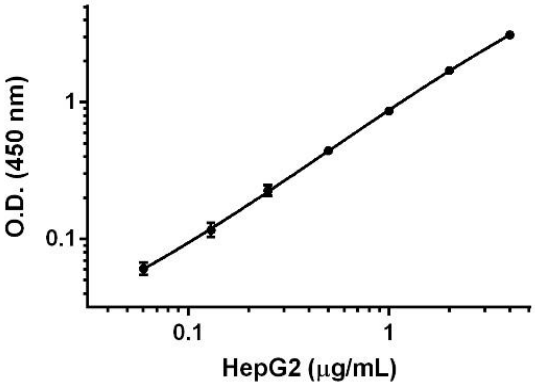


Figure 2. Titration of HepG2 cell extract within the working range of the assay. Background-subtracted data values from triplicate measurements (mean +/- SD) are graphed.

RECOVERY –

Sample Type	Average % Recovery	Range (%)
50% Cell Culture Media (10F HGDMEM)	92.1	85.5 - 95.6
10% Fetal Bovine Serum	79.0	75.7 - 82.0

Linearity of Dilution

Linearity of dilution is determined based on interpolated values from the standard curve. Linearity of dilution defines a sample concentration interval in which interpolated target concentrations are directly proportional to sample dilution.

Native SOD2 was measured in a HepG2 lysate sample in a 2-fold dilution series. Sample dilutions are made in 1X Cell Extraction Buffer PTR.

Dilution Factor	HepG2 Lysate (µg/mL)	Interpolated value (ng/mL)	% Expected Value
Undiluted	4	71.75	100.0
2	2	35.76	99.7
4	1	17.45	97.3
8	0.5	8.92	99.4
16	0.25	4.63	103.2
32	0.125	2.41	107.3
64	0.062	1.25	111.6

17. Assay Specificity

This assay recognizes both native and recombinant human SOD2 protein.

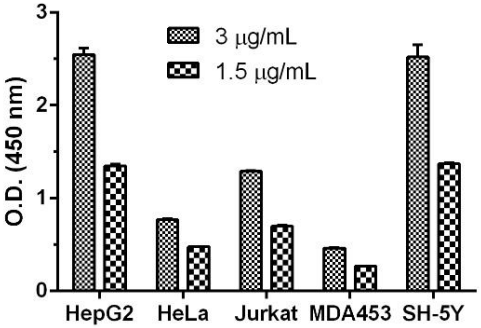


Figure 3. Comparison of SOD2 expression in various cell lines (HepG2, HeLa, Jurkat, MDA-MB-453 (MDA453) and SH-SY5Y (SH-5Y)) using SOD2 Human SimpleStep ELISA Kit. Background-subtracted data values from triplicate measurements of two lysate concentrations (3 and 1.5 µg/mL) are graphed as mean +/- SD.

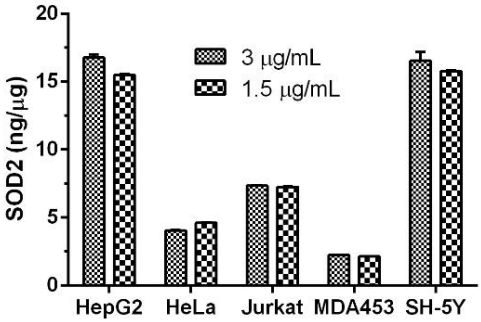


Figure 4. Quantification of SOD2 expression in various cell lines (HepG2, HeLa, Jurkat, MDA-MB-453 (MDA453) and SH-SY5Y (SH-5Y)) using SOD2 Human SimpleStep ELISA Kit. The concentrations of SOD2 were interpolated from data values shown in Figure 3 using SOD2 standard curve, corrected for sample dilution, and graphed in ng of SOD2 per µg of lysate. Note that this result correlates well with Western blot analysis (Figure 5).

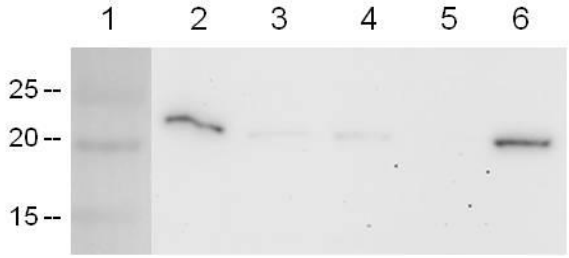


Figure 5. Comparison of SOD2 expression in various cell lines by Western blotting. Cell lysates (20 µg) of HepG2 (lane 2), HeLa (lane 3), Jurkat (lane 4), MDA-MB-453 (lane 5) and SH-SY5Y (lane 6) were analyzed by Western blotting using ab13533 as primary antibody. Lane 1 shows migration of molecular weight marker.

18. Species Reactivity

This kit detects SOD2 in human cell and tissue lysate samples only.

Human serum samples are not compatible with this kit. Plasma samples have not been tested with this kit.

Please contact our Technical Support team for more information.

19. Troubleshooting

Problem	Reason	Solution
Difficulty pipetting lysate; viscous lysate.	Genomic DNA solubilized	Prepare 1X Cell Extraction Buffer PTR (without enhancer). Add enhancer to lysate after extraction.
Poor standard curve	Inaccurate Pipetting	Check pipettes
	Improper standard dilution	Prior to opening, briefly spin the stock standard tube and dissolve the powder thoroughly by gentle mixing
Low Signal	Incubation times too brief	Ensure sufficient incubation times; increase to 2 or 3 hour standard/sample incubation
	Inadequate reagent volumes or improper dilution	Check pipettes and ensure correct preparation
	Incubation times with TMB too brief	Ensure sufficient incubation time until blue color develops prior addition of Stop solution
Large CV	Plate is insufficiently washed	Review manual for proper wash technique. If using a plate washer, check all ports for obstructions.
	Contaminated wash buffer	Prepare fresh wash buffer
Low sensitivity	Improper storage of the ELISA kit	Store your reconstituted standards at -80°C, all other assay components 4°C. Keep TMB Development Solution protected from light.
Precipitate in Diluent	Precipitation and/or coagulation of components within the Diluent.	Precipitate can be removed by gently warming the Diluent to 37°C.

Technical Support

For all technical or commercial enquiries please go to:

www.abcam.com/contactus
www.abcam.cn/contactus (China)
www.abcam.co.jp/contactus (Japan)