DuoSet® IC

Human/Mouse/Rat Total SOD2/Mn-SOD

Catalog Number DYC3419-2
DYC3419-5

For the development of sandwich ELISAs to measure Superoxide Dismutase-2/ Manganese Superoxide Dismutase (SOD2/Mn-SOD) in cell lysates.

This package insert must be read in its entirety before using this product.
For research use only. Not for use in diagnostic procedures.

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MANUFACTURED AND DISTRIBUTED BY:

USA & Canada | R&D Systems, Inc.
614 McKinley Place NE, Minneapolis, MN 55413, USA
TEL: (800) 343-7475 (612) 379-2956 FAX: (612) 656-4400
E-MAIL: info@RnDSystems.com

DISTRIBUTED BY:

UK & Europe | R&D Systems Europe, Ltd.
19 Barton Lane, Abingdon Science Park, Abingdon OX14 3NB, UK
TEL: +44 (0)1235 529449 FAX: +44 (0)1235 533420
E-MAIL: info@RnDSystems.co.uk

China | R&D Systems China Co., Ltd.
24A1 Hua Min Empire Plaza, 726 West Yan An Road, Shanghai PRC 200050
TEL: +86 (21) 52380373 FAX: +86 (21) 52371001
E-MAIL: info@RnDSystemsChina.com.cn

PRINCIPLE OF THE ASSAY

This DuoSet® IC ELISA contains the basic components required for the development of sandwich ELISAs to measure Superoxide Dismutase-2 (SOD2), also known as Manganese Superoxide Dismutase (Mn-SOD), in cell lysates. An immobilized capture antibody specifically binds human/mouse/rat SOD2. After washing away unbound material, a biotinylated detection antibody specific for human/mouse/rat SOD2 is used to detect captured protein utilizing a standard Streptavidin-HRP format.

MATERIALS PROVIDED & STORAGE CONDITIONS

Store the unopened kit at 2-8 °C. Do not use past kit expiration date.

DESCRIPTION	PART #	CATALOG # DYC3419-2	CATALOG # DYC3419-5	STORAGE OF OPENED/ RECONSTITUTED MATERIAL
Human/Mouse/Rat Total SOD2/Mn-SOD Capture Antibody	842923	1 vial	2 vials	Store for up to 1 month at 2-8 °C or aliquot and store at ≤ -20 °C for up to 3 months in a manual defrost freezer.*
Human/Mouse/Rat Total SOD2/Mn-SOD Detection Antibody	842924	1 vial	2 vials	
Human/Mouse/Rat Total SOD2/Mn-SOD Standard	842925	3 vials	5 vials	**Use within one hour of reconstitution. Use a fresh standard for each assay.**
Streptavidin-HRP	890803	1 vial	1 vial	Store for up to 1 month at 2-8 °C. **DO NOT FREEZE.**

* Provided this is within the expiration date of the kit.

DYC3419-2 contains sufficient materials to run ELISAs on at least two 96 well plates. †
DYC3419-5 contains sufficient materials to run ELISAs on at least five 96 well plates. †

† Provided the following conditions are met:
- The reagents are prepared as described in this package insert.
- The assay is run as described in the General ELISA Protocol on page 6.
- The recommended microplates, buffers, diluents, substrates, and solutions are used.

OTHER MATERIALS REQUIRED

- Aprotinin (Sigma # A6279)
- Leupeptin (Tocris # 1167)
- Pepstatin (Tocris # 1190)
- Phenylmethylsulfonylfluoride (PMSF) (Sigma # P7626)
- Sodium Orthovanadate (Na3VO4) (Sigma # S6508), activated
- Sodium Pyrophosphate (Na4P2O7) (Sigma # P8010)
- Sodium Fluoride (NaF) (Sigma # 201154)
- Sodium Azide (NaN3) (Sigma # S2002)
- TritonTM X-100 (Sigma # T9284)
- Urea
- Pipettes and pipette tips
- Deionized or distilled water
- 96 well microplates (R&D Systems Catalog # DY990)
- Plate sealers (R&D Systems, Catalog # DY992)
- Squirt bottle, manifold dispenser, or automated microplate washer

SOLUTIONS REQUIRED

PBS - 137 mM NaCl, 2.7 mM KCl, 8.1 mM Na_2HPO_4, 1.5 mM KH_2PO_4, pH 7.2-7.4, 0.2 µm filtered (R&D Systems, Catalog # DY006).

Wash Buffer - 0.05% Tween® 20 in PBS, pH 7.2-7.4 (R&D Systems, Catalog # WA126).

Block Buffer - 1% BSA*, 0.05% NaN_3 in PBS, pH 7.2-7.4.

IC Diluent #1 - 1% BSA* in PBS, pH 7.2-7.4, 0.2 µm filtered.

IC Diluent #8** - 1 mM EDTA, 0.5% Triton X-100, 5 mM NaF in PBS, pH 7.2-7.4.

Note: *IC Diluent #8 is also the base diluent for IC Diluent #3, IC Diluent #7, and Lysis Buffer #6. Approximately 50 mL of this diluent is required to run the assay on one plate.*

IC Diluent #3** - 1 mM EDTA, 0.5% Triton X-100, 5 mM NaF, 1 M urea in PBS, pH 7.2-7.4.

IC Diluent #7** - 1 mM EDTA, 0.5% Triton X-100, 5 mM NaF, 6 M urea in PBS, pH 7.2-7.4.

Lysis Buffer #6** - 1 mM EDTA, 0.5% Triton X-100, 5 mM NaF, 6 M urea, 1 mM activated sodium orthovanadate, 2.5 mM sodium pyrophosphate, 10 µg/mL Leupeptin, 10 µg/mL Pepstatin, 100 µM PMSF, 3 mg/mL Aprotinin in PBS, pH 7.2-7.4.

Substrate Solution - 1:1 mixture of Color Reagent A (H_2O_2) and Color Reagent B (Tetramethylbenzidine) (R&D Systems, Catalog # DY999).

Stop Solution - 2 N H_2SO_4 (R&D Systems, Catalog # DY994).

*The use of R&D Systems Reagent Diluent Concentrate 2 (Catalog # DY995) or Millipore Bovine Serum Albumin, Fraction V, Protease free (Catalog # 82-045) is recommended. All buffers containing BSA must be stored at 2-8°C.

**Sample Diluent Concentrate 1 (5X) (R&D Systems, Catalog # DYC001), prepared as described in the DYC001 insert.

REAGENT PREPARATION

Bring all reagents to room temperature before use.

Human/Mouse/Rat Total SOD2/Mn-SOD Capture Antibody Part 842923) - Each vial contains 180 µg/mL of mouse anti-human SOD2 antibody when reconstituted with 200 mL of PBS. After reconstitution, store at 2-8°C for up to 30 days or aliquot and store at ≤ -20°C in a manual defrost freezer or at ≤ -70°C for up to 3 months.*

Human/Mouse/Rat Total SOD2/Mn-SOD Detection Antibody (Part 842924) - Each vial contains 1.8 µg/mL of biotinylated goat anti-human SOD2 antibody when reconstituted with 1.0 mL of IC Diluent #1. After reconstitution, store at 2-8°C for up to 30 days or aliquot and store at ≤ -20°C in a manual defrost freezer or at ≤ -70°C for up to 3 months.*

Human/Mouse/Rat Total SOD2/Mn-SOD Standard (Part 842925) - Each vial contains 230 ng/mL of recombinant human SOD2 when reconstituted with 500 µL of IC Diluent #7. **Use within one hour after reconstitution. A fresh standard should be used for each assay.** Immediately before use, an initial 6-fold dilution should be made in IC Diluent #8. Additional dilutions should be made in IC Diluent #3. A seven point standard curve using 2-fold serial dilutions and a high standard of 20,000 pg/mL is recommended.

Streptavidin-HRP (Part 890803) - 1 mL of Streptavidin conjugated to horseradish-peroxidase. Store at 2-8°C. **DO NOT FREEZE.** Immediately before use, dilute the Streptavidin-HRP to the working concentration specified on the vial label using IC Diluent #1.

*Provided this is within the expiration date of the kit

PREPARATION OF SAMPLES

Cell Lysates - Rinse cells two times with PBS, making sure to remove any remaining PBS after the second rinse. Solubilize cells at 1 x 10^7 cells/mL in Lysis Buffer #6 and allow samples to sit on ice for 15 minutes. Assay immediately or store at ≤ -70°C. Before use, centrifuge samples at 2000 x g for 5 minutes and transfer the supernate to a clean test tube. Sample protein concentration may be quantified using a total protein assay. For assaying, dilute lysates 6-fold with IC Diluent #8 and make further serial dilutions in IC Diluent #3.

Note: *The final concentration of urea in all samples and standards should be 1 M prior to addition to the plate.*

PRECAUTION

The Stop Solution provided with this kit is an acid solution.

Some components in this kit contain a preservative which may cause an allergic skin reaction. Avoid breathing mist.

Wear protective gloves, clothing, eye, and face protection. Wash hands thoroughly after handling. Please refer to the MSDS on our website prior to use.

TECHNICAL HINTS AND LIMITATIONS

- This DuoSet® IC ELISA should not be used beyond the expiration date on the kit label.
- Individual results may vary due to differences in technique, plasticware, and water sources.
- It is important that the diluents selected for reconstitution and for dilution of the control reflect the environment of the samples being measured. The diluent suggested in this protocol should be suitable for most cell lysates.
- The type of enzyme and substrate and the concentrations of capture/detection antibodies used can be varied to create an immunoassay with a different sensitivity and dynamic range. A basic understanding of immunoassay development is required for the successful use of these reagents in immunoassays.
- A thorough and consistent wash technique is essential for proper assay performance. Wash Buffer should be dispensed forcefully and removed completely from the wells by aspiration or decanting. Remove any remaining Wash Buffer by inverting the plate and blotting it against clean paper towels.
- Use a fresh reagent reservoir and pipette tips for each step.
- It is recommended that all standards and samples be assayed in duplicate.
- Avoid microbial contamination of reagents and buffers. This may interfere with the sensitivity of the assay. Buffers containing protein should be made under aseptic conditions and stored at 2-8°C or be prepared fresh daily.

GENERAL ELISA PROTOCOL

Plate Preparation

1. Dilute the Capture Antibody to a working concentration of 1.0 µg/mL in PBS, without carrier protein. Immediately coat a 96 well microplate with 100 µL per well of the diluted Capture Antibody. Seal the plate and incubate overnight at room temperature.
2. Aspirate each well and wash with Wash Buffer, repeating the process two times for a total of 3 washes. Wash by filling each well with Wash Buffer (400 µL) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each step is essential for good performance. After the last wash, remove any remaining Wash Buffer by aspirating or by inverting the plate and blotting it against clean paper towels.
3. Block plates by adding 300 µL of Block Buffer to each well. Incubate at room temperature for 1-2 hours.
4. Repeat the aspiration/wash as in step 2. The plates are now ready for sample addition.

Assay Procedure

1. Add 100 µL of sample or standards in IC Diluent #3 per well. Use IC Diluent #3 as the zero standard. Cover with a plate sealer and incubate 2 hours at room temperature.
   Note: *A seven point standard curve using 2-fold serial dilutions and a high standard of 20,000 pg/mL is recommended.*
2. Repeat the aspiration/wash as in step 2 of Plate Preparation.
3. Immediately before use, dilute the Detection Antibody to working concentration of 50 ng/mL using IC Diluent #1. Prepare only as much Detection Antibody as required to run each assay. Add 100 µL of the diluted Detection Antibody to each well. Cover with a new plate sealer and incubate 2 hours at room temperature.
4. Repeat the aspiration/wash as in step 2 of Plate Preparation.
5. Add 100 µL of the diluted Streptavidin-HRP to each well. Incubate for 20 minutes at room temperature. Avoid placing the plate in direct light.
6. Repeat the aspiration/wash as in step 2 of the Plate Preparation.
7. Add 100 µL of Substrate Solution to each well. Incubate for 20 minutes at room temperature. Avoid placing the plate in direct light.
8. Add 50 µL of Stop Solution to each well. Gently tap the plate to ensure thorough mixing.
9. Determine the optical density of each well immediately, using a microplate reader set to 450 nm. If wavelength correction is available, set to 540 nm or 570 nm. If wavelength correction is not available, subtract readings at 540 nm or 570 nm from the readings at 450 nm. This subtraction will correct for optical imperfections in the plate. Readings made directly at 450 nm without correction may be higher and less accurate.

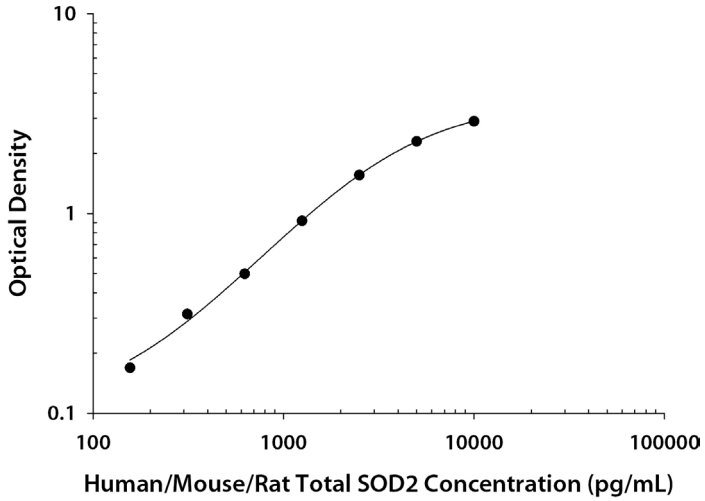
CALCULATION OF RESULTS

Average the duplicate readings for each standard and sample, then subtract the average zero standard optical density (O.D.). Results may be normalized to total protein or cell number.

Create a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the log of the human/mouse/rat SOD2 concentrations versus the log of the O.D. and the best fit line can be determined by regression analysis. This procedure will produce an adequate but less precise fit of the data.

TYPICAL DATA

A standard curve should be generated for each set of samples assayed. The graph below represents typical data generated when using the Human/Mouse/Rat Total SOD2/Mn-SOD DuoSet® IC ELISA. The standard curve was calculated using a computer generated 4-PL curve-fit. This standard curve is for demonstration purposes only.



CALIBRATION

The Human/Mouse/Rat Total SOD2/Mn-SOD DuoSet® IC ELISA is calibrated against a highly purified *E. coli*-expressed recombinant human SOD2 produced at R&D Systems. Samples containing natural SOD2 showed linear dilution parallel to the standard curve obtained using the Human/Mouse/Rat Total SOD2/Mn-SOD Standard. These results indicate that O.D. values from this DuoSet® IC ELISA can be used to determine the relative concentration of SOD2 in natural samples.

SPECIFICITY

The Human/Mouse/Rat Total SOD2/Mn-SOD DuoSet® IC ELISA specifically recognizes SOD2. Specificity was demonstrated by Western blot analysis of the protein bound by the capture antibody supplied in the ELISA.

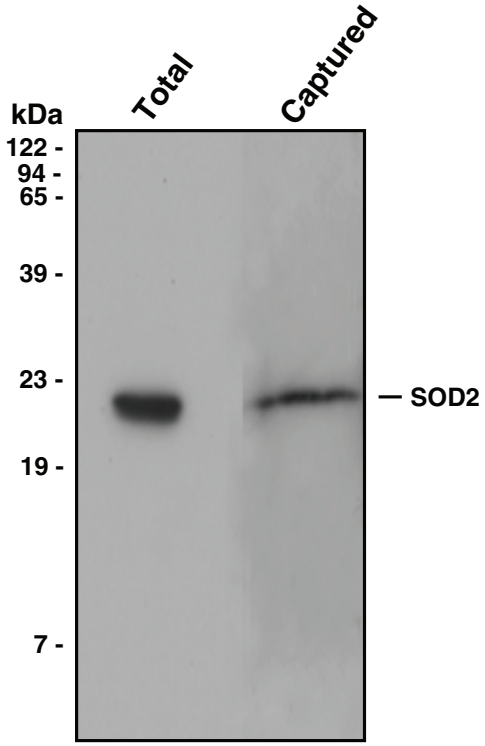


Figure 1: Lysates prepared from HepG2 human hepatocellular carcinoma cells were incubated in wells coated with Human/Mouse/Rat Total SOD2/Mn-SOD Capture Antibody. Unbound material was removed by washing and bound material was solubilized in SDS gel sample buffer. The same lysate and captured protein were electrophoresed, transferred to a PVDF membrane, and immunoblotted with an SOD2 polyclonal antibody (R&D Systems, Catalog # AF3419). Only the band corresponding to SOD2 was detected in captured material.

To further determine specificity, recombinant human SOD1 and recombinant human SOD3 were assayed at 200 ng/mL and did not cross-react or interfere in the assay.

QUANTIFICATION

Amounts of SOD2, as quantified by the Human/Mouse/Rat Total SOD2/Mn-SOD DuoSet® IC ELISA, are consistent with the relative amounts of SOD2 determined by qualitative Western blot analysis.

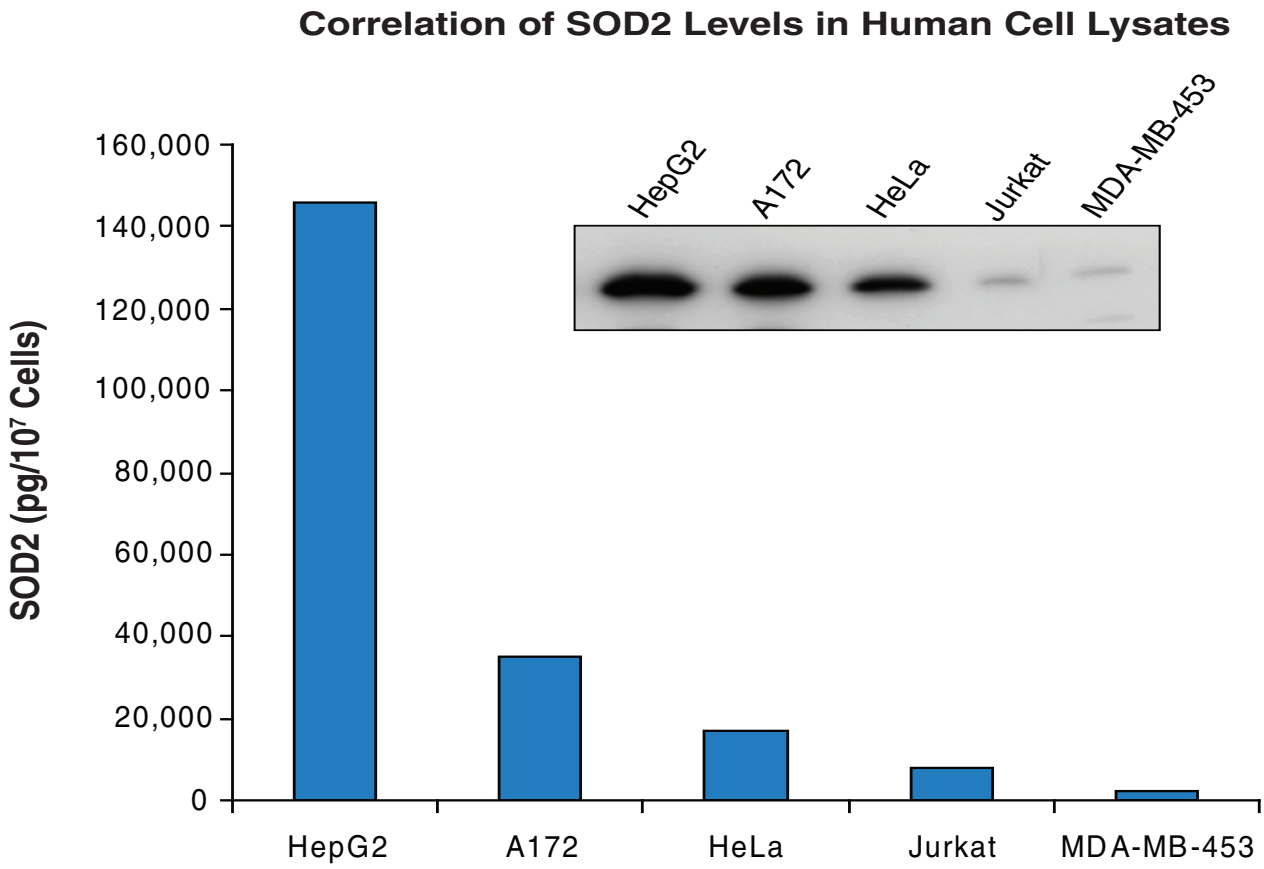


Figure 2: Lysates prepared from HepG2 human hepatocellular carcinoma cells, A172 human glioblastoma cells, HeLa human cervical epithelial carcinoma cells, Jurkat human acute T cell leukemia cells, and MDA-MB-453 human breast cancer cells were quantified with this DuoSet® IC ELISA. The same lysates were immunoblotted (inset) with an anti-SOD2 polyclonal antibody. The DuoSet® IC ELISA results correlate well with the relative amounts of SOD2 detected by Western blot.

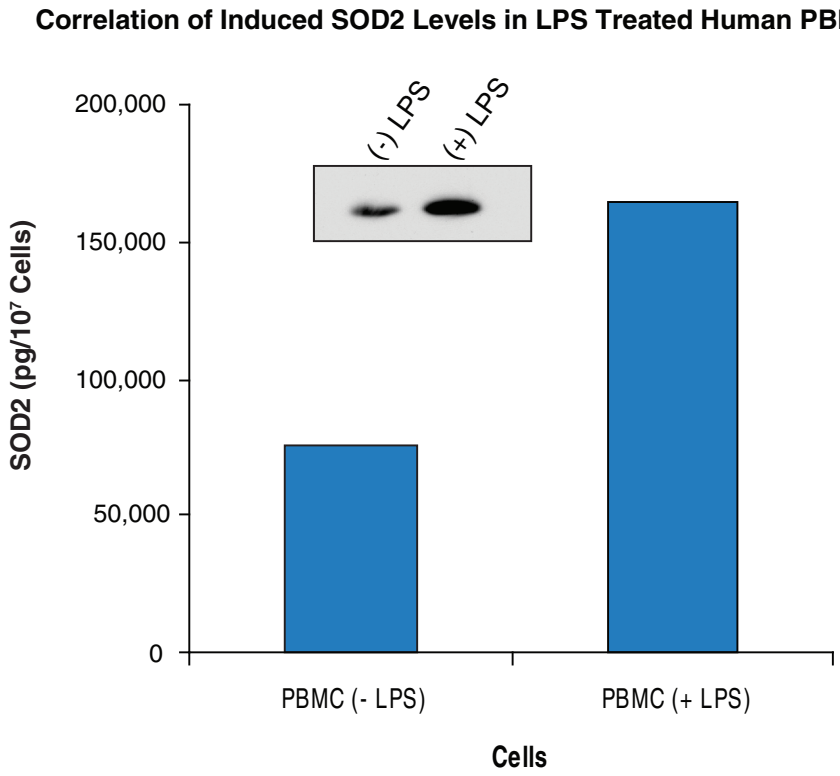


Figure 3: Human peripheral blood mononuclear cells (PBMCs) were cultured for 24 hours in the absence or presence of 1 mM lipopolysaccharide (LPS). The lysates were quantified with this DuoSet® IC ELISA. The same lysates were immunoblotted (inset) with an anti-SOD2 polyclonal antibody. The DuoSet® IC ELISA results correlate well with the relative amounts of SOD2 detected by Western blot.

Amounts of SOD2 as quantified by the Human/Mouse/Rat Total SOD2/Mn-SOD DuoSet® IC ELISA are consistent with the relative amounts of SOD2 determined by qualitative Western blot analysis.

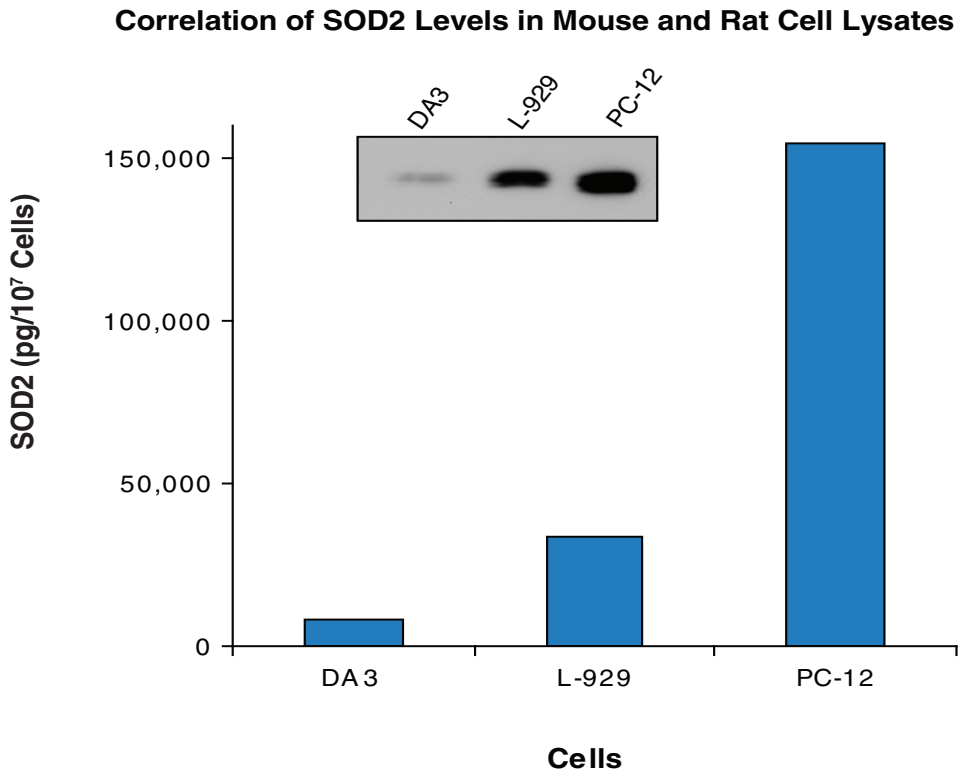


Figure 4: Lysates prepared from DA3 mouse myeloma cells, L-929 mouse fibroblast cells, and PC-12 rat adrenal pheochromocytoma cells were quantified with this DuoSet® IC ELISA. The same lysates were immunoblotted (inset) with an anti-SOD2 polyclonal antibody. The DuoSet® IC ELISA results correlate well with the relative amounts of SOD2 detected by Western blot.