



Spp1 (Mouse) ELISA Kit

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96 assays

Version: 04

Intended for research use only

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Introduction

Intended Use

For the quantitation of Mouse Spp1 concentrations in serum, plasma, cell culture supernates, urine.

Background

Osteopontin (OPN) also called urinary stone protein, secreted phosphoprotein 1 (SPP1), bone sialoprotein, and early T lymphocyte activation 1 (ETA1). Osteopontin is a phosphorylated glycoprotein secreted to the mineralizing extracellular matrix by osteoblasts during bone development. It is believed to facilitate the attachment of osteoblasts and osteoclasts to the extracellular matrix, allowing them to perform their respective functions during osteogenesis. Osteopontin is presumably involved in stone formation as stone matrix. The deduced protein sequence reveals a 317-amino acid protein (34,982 Da) containing a 16-amino acid hydrophobic signal sequence and a 33,352-Da protein destined to undergo extensive post-translational modifications before being secreted from the cell. The gene is located on human chromosome 4. The standard product used in this kit is recombinant mouse OPN, consisting of 284 amino acid sequence with the molecular mass of 31.5 KDa. As a result of glycosylation, the molecular mass is 65 KDa.

Principle of the Assay

The Spp1 (Mouse) ELISA Kit is a solid phase immunoassay specially designed to measure Mouse Spp1 with a 96-well strip plate that is pre-coated with antibody specific for Spp1. The detection antibody is a biotinylated antibody specific for Spp1. The capture antibody is monoclonal antibody from rat, the detection antibody is polyclonal antibody from goat. The kit contains recombinant Mouse Spp1 with immunogen: Expression system for standard: NSO; Immunogen sequence: L17-N294. The kit is analytically validated with ready to use reagents.

To measure Mouse Spp1, add standards and samples to the wells, then add the biotinylated detection antibody. Wash the wells with PBS or TBS buffer, and add Avidin-Biotin-Peroxidase Complex (ABC-HRP). Wash away the unbound ABC-HRP with PBS or TBS buffer and add TMB. TMB is substrate to HRP and will be catalyzed to produce a blue color product, which changes into yellow after adding acidic stop solution. The density of the yellow product is linearly proportional to Mouse Spp1 in the sample. Read the density of the yellow product in each well using a plate reader, and benchmark the sample wells' readings against the standard curve to determine the concentration of Mouse Spp1 in the sample.

General Information

Materials Supplied

List of component

Component	Amount
Anti-Mouse Spp1 Pre-coated 96-well strip microplate	96 (8x12) wells
Mouse Spp1 Standard	10 ng/tube x 2
Mouse Spp1 Biotinylated antibody (100x)	130 µL
Avidin-Biotin-Peroxidase Complex (100x)	130 µL
Sample Diluent	30 mL
Antibody Diluent	12 mL
Avidin-Biotin-Peroxidase Diluent	12 mL
Color Developing Reagent (TMB)	10 mL
Stop solution	10 mL
Wash Buffer Powder	1 Pack
Plate Sealers	4 slides

Storage Instruction

Store at 4°C for 6 months, at -20°C for 12 months. Avoid multiple freeze-thaw cycles.

Materials Required but Not Supplied

- ✓ Microplate Reader capable of reading absorbance at 450 nm.
- ✓ Automated plate washer (optional)
- ✓ Pipettes and pipette tips capable of precisely dispensing 0.5 µL through 1 mL volumes of aqueous solutions.
- ✓ Multichannel pipettes are recommended for large amount of samples.
- ✓ Deionized or distilled water.
- ✓ 500 mL graduated cylinders.
- ✓ Test tubes for dilution.

Precautions for Use

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

- ✓ Notice Before Application

Please read the following instructions before starting the experiment.

1. To inspect the validity of experiment operation and the appropriateness of sample dilution proportion, pilot experiment using standards and a small number of samples is recommended.
2. Before using the Kit, spin tubes and bring down all components to the bottom of tubes.
3. Don't let 96-well plate dry, for dry plate will inactivate active components on plate.
4. Don't reuse tips and tubes to avoid cross contamination.
5. Avoid using the reagents from different batches together.

Assay Protocol

Reagent Preparation

- ✓ Bring all reagents to 37°C prior to use. The assay can also be done at room temperature however we recommend doing it at 37°C for best consistency with our QC results. Also the TMB incubation time estimate (20-25 min) is based on 37°C.
 - Wash buffer - Dissolve the wash buffer powder in 1000 mL of deionized water. Excess wash buffer can be stored for up to one week at 4°C.
 - Biotinylated Anti-Mouse Spp1 antibody - It is recommended to prepare this reagent immediately prior to use by diluting the Mouse Spp1 Biotinylated antibody (100x) 1:100 with Antibody Diluent. Prepare 100 µL by adding 1 µL of Biotinylated antibody (100x) to 99 µL of Antibody Diluent for each well. Mix gently and thoroughly and use within 2 hours of generation.
 - Avidin-Biotin-Peroxidase Complex - It is recommended to prepare this reagent immediately prior to use by diluting the Avidin-Biotin-Peroxidase Complex (100x) 1:100 with Avidin-Biotin-Peroxidase Diluent. Prepare 100 µL by adding 1 µL of Avidin-Biotin-Peroxidase Complex (100x) to 99 µL of Avidin-Biotin-Peroxidase Diluent for each well. Mix gently and thoroughly and use within 2 hours of generation.
 - Mouse Spp1 Standard - It is recommended that the standards be prepared no more than 2 hours prior to performing the experiment. Use one 10 ng of lyophilized Mouse Spp1 standard for each experiment. Gently spin the vial prior to use. Reconstitute the standard to a stock concentration of 10 ng/mL using 1 mL of sample diluent. Allow the standard to sit for a minimum of 10 minutes with gentle agitation prior to making dilutions.
 - Microplate - The included microplate is coated with capture antibodies and ready-to-use. It does not require additional washing or blocking. The unused well strips should be sealed and stored in the original packaging.

- ✓ Dilution of Mouse Spp1 Standard
 1. Number tubes 1-8. Final Concentrations to be Tube # 1 –10000 pg/mL, #2 –5000 pg/mL, #3 – 2500 pg/mL, #4 – 1250 pg/mL, #5 – 625 pg/mL, #6 – 312.5 pg/mL, #7 – 156.25 pg/mL, #8 – 0.0 (Blank).
 2. For standard #1, add 1000 µL of undiluted standard stock solution to tube #1.
 3. Add 300 µL of sample diluent to tubes # 2-7.
 4. To generate standard #2, add 300 µL of standard #1 from tube #1 to tube #2 for a final volume of 600 µL. Mix thoroughly.
 5. To generate standard #3, add 300 µL of standard #2 from tube #2 to tube #3 for a final volume of 600 µL. Mix thoroughly.
 6. Continue the serial dilution for tube #4-7.
 7. Tube #8 is a blank standard to be used with every experiment.

Sample Preparation

✓ Sample Preparation and Storage

These sample collection instructions and storage conditions are intended as a general guideline and the sample stability has not been evaluated.

- Cell culture supernates: Clear sample of particulates by centrifugation, assay immediately or store samples at -20°C.
- Serum: Use a serum separator tube (SST) and allow serum to clot at room temperature for about four hours. Then, centrifuge for 15 min at approximately 1,000 x g. assay immediately or store samples at -20°C.
- Plasma: Collect plasma using as an anticoagulant. Centrifuge for 15 min at approximately 1,000 x g. Assay immediately or store samples at -20°C.

*Note: it is important to not use anticoagulants other than the ones described above to treat plasma for other anticoagulants could block the antibody binding site.

- Urine: Collect the first urine of the day, micturate directly into a sterile container. Remove impurities by centrifugation, assay immediately or aliquot and store samples at -20°C.

✓ Sample Dilution Guideline

The target protein concentration should be estimated and appropriate sample dilutions should be selected such that the final protein concentration lies near the middle of the linear dynamic range of the assay.

It is recommended to prepare 150 µL of sample for each replicate to be assayed. The samples should be diluted with sample diluent and mixed gently.

Assay Procedure

It is recommended that all reagents and materials be equilibrated to 37°C/room temperature prior to the experiment (see Reagent Preparation if you have missed this information).

1. Prepare all reagents and working standards as directed previously.
2. Remove excess microplate strips from the plate frame and seal and store them in the original packaging.
3. Add 100 μ L of the standard, samples, or control per well. Add 100 μ L of the sample diluent buffer into the control well (Zero well). At least two replicates of each standard, sample, or control is recommended.
4. Cover with the plate sealer provided and incubate for 120 minutes at RT (or 90 min. at 37 °C).
5. Remove the cover and discard the liquid in the wells into an appropriate waste receptacle. Invert the plate on the benchtop onto a paper towel and tap the plate to gently blot any remaining liquid. It is recommended that the wells are not allowed to completely dry at any time.
6. Add 100 μ L of the prepared 1x Biotinylated Anti-Mouse Spp1 antibody to each well.
7. Cover with plate sealer and incubate for 90 minutes at RT (or 60 minutes at 37°C).
8. Wash the plate 3 times with the 1x wash buffer.
 - a. Discard the liquid in the wells into an appropriate waste receptacle. Then, invert the plate on the benchtop onto a paper towel and tap the plate to gently blot any remaining liquid. It is recommended that the wells are not allowed to completely dry at any time.
 - b. Add 300 μ L of the 1x wash buffer to each assay well. (For cleaner background incubate for 60 seconds between each wash).
 - c. Repeat steps a-b 2 additional times.
9. Add 100 μ L of the prepared 1x Avidin-Biotin-Peroxidase Complex into each well and incubate for 40 minutes at RT (or 30 minutes at 37°C).
10. Wash the plate 5 times with the 1x wash buffer.
 - a. Discard the liquid in the wells into an appropriate waste receptacle. Then, invert the plate on the benchtop onto a paper towel and tap the plate to gently blot any remaining liquid. It is recommended that the wells are not allowed to completely dry at any time.
 - b. Add 300 μ L of the 1x wash buffer to each assay well. (For cleaner background incubate for 60 seconds between each wash).
 - c. Repeat steps a-b 4 additional times.
11. Add 90 μ L of Color Developing Reagent to each well and incubate in the dark for 30 minutes at RT (or 25-30 minutes at 37°C). (The optimal incubation time must be empirically determined. A guideline to look for is blue shading the top four standard wells, while the remaining standards remain clear.)
12. Add 100 μ L of Stop Solution to each well. The color should immediately change to yellow.
13. Within 30 minutes of stopping the reaction, the O.D. absorbance should be read with a microplate reader at 450 nm.

Data Analysis

Calculation of Results

Average the duplicate readings for each standard, sample, and control. Subtract the average zero standard O.D. reading.

It is recommended that a standard curve be created using computer software to generate a four parameter logistic (4-PL) curve-fit.

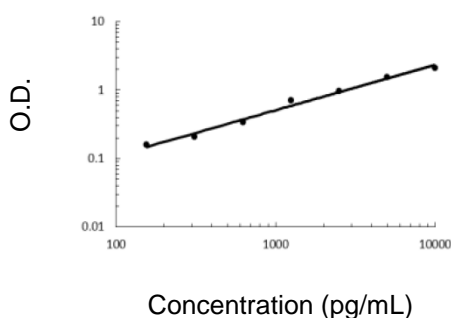
Alternatively, plot the mean absorbance for each standard against the concentration. The measured concentration in the sample can be interpolated by using linear regression of each average relative OD against the standard curve generated using curve fitting software. This will generate an adequate but less precise fit of the data.

For diluted samples, the concentration reading from the standard curve must be multiplied by the dilution factor.

✓ Spp1 (Mouse) ELISA Kit Standard Curve Example

Highest O.D. value might be higher or lower than in the example. The experiment result is statistically significant if the highest O.D. value is no less than 1.0.

Concentration (pg/mL)	0	156	312	625	1250	2500	5000	10000
O.D	0.042	0.158	0.208	0.336	0.706	0.972	1.547	2.129



A standard curve is provided for demonstration only. A standard curve should be generated for each set of samples assayed.

Performance Characteristics

✓ Detection Range: 156 - 10000 pg/mL

✓ Sensitivity: < 10 pg/mL

*The sensitivity or the minimum detectable dose (MDD) is the lower limit of target protein that can be detected by the kit. It is determined by adding two standard deviations to the mean O.D. value of twenty (20) blank wells and calculating the corresponding concentration.

✓ Specificity: Natural and recombinant Mouse Spp1

✓ Cross-reactivity: There is no detectable cross-reactivity with other relevant proteins.

✓ Intra/Inter Assay Variability

- Intra-Assay Precision (Precision within an assay)

Three samples of known concentration were tested on one plate to assess intra-assay precision.

- Inter-Assay Precision (Precision across assays)

Three samples of known concentration were tested in separate assays to assess inter-assay precision.

	Intra-Assay Precision			Inter-Assay Precision		
Sample	1	2	3	1	2	3
n	16	16	16	24	24	24
Mean (pg/mL)	306	1986	5401	308	2000	4959
Standard deviation	14.68	117.17	405.07	15.09	142	447.1
CV (%)	4.8%	7.5%	7.5%	4.9%	7.1%	9%

✓ Reproducibility

To assay reproducibility, three samples with differing target protein concentrations were assayed using four different lots.

Lots	Lot1 (pg/mL)	Lot2 (pg/mL)	Lot3 (pg/mL)	Lot4 (pg/mL)	Mean (pg/mL)	Standard Deviation	CV (%)
Sample 1	306	280	315	325	306	16.71	5.4%
Sample 2	1986	2147	1896	1939	1992	94.98	4.7%
Sample 3	5401	5537	5315	5912	5541	228.21	4.1%

*number of samples for each test n=16.

Resources

Plate Layout

12								
11								
10								
9								
8								
7								
6								
5								
4								
3								
2								
1								
	A	B	C	D	E	F	G	H