Quantikine® ELISA

Mouse/Rat WISP-1/CCN4 Immunoassay

Catalog Number MWSP10

For the quantitative determination of mouse or rat Wnt-Induced Secreted Protein-1 (WISP-1) concentrations in cell culture supernates, tissue lysates, serum, plasma, and urine.

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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INTRODUCTION

WISP-1 (Wnt-Induced Secreted Protein-1), also known as CCN4, is a 40 kDa secreted heparin-binding glycoprotein that is a member of the CCN (Cysteine-rich 61, Connective tissue growth factor, and Nephroblastoma overexpressed) family of matricellular proteins. Other family members include Cyr61/CCN1, CTGF/CCN2, NOV/CCN3, WISP2/CCN5 and WISP3/CCN6 (1-5). Like most CCN proteins, WISP-1 is a modular protein containing IGFBP-like, von Willebrand factor type C (VWC), TSP type I, and C-terminal cysteine knot domains (1-4). The VWC module mediates interaction with proteins such as BMPs and TGF-β, the TSP domain binds sulfated glycoconjugates, and the cysteine knot mediates dimerization and receptor binding (3-5). It is likely that WISP-1 normally circulates as a homodimer (4, 5). Mature mouse WISP-1 shares 98%, 84%, and 85% amino acid identity with rat, canine and human WISP-1, respectively. The WISP1v isoform, which lacks the VWC domain, is identified as an oncoprotein human scirrhous gastric carcinoma, but is also upregulated in chondrocytes at terminal differentiation (5, 6). WISP-1 is expressed by differentiating osteoblasts and fibroblastic stroma, including tumor stroma (2, 5-11). Its highest expression during development is found in bone and lung (4). TGF-β, TNF-α, BMP-2, nitric oxide and some Wnts, such as Wnt1 and Wnt3a, induce WISP-1 expression, while cortisol suppresses WISP-1 expression (2, 4, 9-13).

WISP-1 promotes bone cell formation and contributes to skeletogenesis and fracture healing (2, 4, 7, 11). It interacts with perichondral mesenchyme and undifferentiated chondrocytes, promoting proliferation but repressing further chondrocytic differentiation (7). It regulates TGF-β signaling in osteoblastic cells (11). It also enhances the expression of integrin α5β1, and its binding to α5β1 allows WISP-1 to potentiate BMP-2-induced osteoblastic differentiation (7, 8, 11). Its expression in synovium and cartilage is increased in osteoarthritis, and it potentially contributes to osteoarthritis by enhancing matrix metalloproteinase (MMP) expression by synovial cells and chondrocytes (14). It binds dermatan sulfate proteoglycans such as decorin and biglycan in the extracellular matrix, and can antagonize the effects of biglycan on osteogenic cells (4, 15, 16).

WISP-1 and/or WISP1v overexpression in tumors or surrounding stroma is identified as oncogenic in many cancers, where it may accelerate cell growth and increase motility by upregulating MMP expression (2, 5, 12, 19-23). It has anti-apoptotic activity by activating PI 3-Kinase/Akt signaling pathways and antagonizing the p53 tumor suppressor pathway (24, 25). However, WISP-1 can also act as a tumor suppressor. It can suppress melanoma growth and metastasis, and its overexpression in lung cancer cells correlates with lower motility and invasion, probably mediated by integrin signaling (1, 17, 18). In non-cancerous tissues, enhanced WISP-1 expression is found in chemotherapy- or infarct-induced cardiac damage, Alzheimers disease and other neurodegenerative disorders, where it is anti-apoptotic and cytoprotective (10, 25-27). Enhanced expression of WISP-1 also contributes to cell proliferation and collagen synthesis in cardiomyocyte hypertrophy, ventilator-induced lung injury, and idiopathic pulmonary fibrosis (28-30).

The Quantikine® Mouse/Rat WISP-1/CCN4 Immunoassay is a 4.5 hour solid-phase ELISA designed to measure mouse and rat WISP-1 in mouse or rat cell culture supernates, tissue lysates, serum, plasma, and urine. It contains NS0-expressed recombinant mouse WISP-1 and antibodies raised against the recombinant factor. This immunoassay has been shown to accurately quantitate the recombinant mouse WISP-1. Results obtained using natural mouse or rat WISP-1 showed dose response curves that were parallel to the standard curves obtained using the Quantikine® kit standards. These results indicate that this kit can be used to determine relative mass values for natural mouse and rat WISP-1.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for mouse/rat WISP-1 has been pre-coated onto a microplate. Standards, control, and samples are pipetted into the wells and any WISP-1 present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for mouse/rat WISP-1 is added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, a substrate solution is added to the wells. The enzyme reaction yields a blue product that turns yellow when the Stop Solution is added. The intensity of the color measured is in proportion to the amount of WISP-1 bound in the initial step. The sample values are then read off the standard curve.

LIMITATIONS OF THE PROCEDURE

- FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
- The kit should not be used beyond the expiration date on the kit label.
- Do not mix or substitute reagents with those from other lots or sources.
- Samples must be pipetted within 15 minutes.
- If samples generate values higher than the highest standard, further dilute the samples with calibrator diluent and repeat the assay.
- Any variation in diluent, operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.
- Variations in sample collection, processing, and storage may cause sample value differences.
- This assay is designed to eliminate interference by other factors present in biological samples. Until all factors have been tested in the Quantikine® Immunoassay, the possibility of interference cannot be excluded.

TECHNICAL HINTS

- When mixing or reconstituting protein solutions, always avoid foaming.
- To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.
- To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
- Substrate Solution should remain colorless until added to the plate. Keep Substrate Solution protected from light. Substrate Solution should change from colorless to gradations of blue.
- Stop Solution should be added to the plate in the same order as the Substrate Solution. The color developed in the wells will turn from blue to yellow upon addition of the Stop Solution.

MATERIALS PROVIDED & STORAGE CONDITIONS

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

PART	PART #	DESCRIPTION	STORAGE OF OPENED/ RECONSTITUTED MATERIAL
Mouse/Rat WISP-1 Microplate	894360	96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody specific for mouse/rat WISP-1.	Return unused wells to the foil pouch containing the desiccant pack. Reseal along entire edge of the zip-seal. May be stored for up to 1 month at 2-8 °C.*
Mouse/Rat WISP-1 Standard	894362	2 vials of recombinant mouse WISP-1 in a buffered protein base with preservatives; lyophilized. *Refer to the vial label for reconstitution volume.*	Use a new standard and control for each assay. Discard after use.
Mouse/Rat WISP-1 Control	894363	2 vials of recombinant mouse WISP-1 in a buffered protein base with preservatives; lyophilized. The assay value of the control should be within the range specified on the label.	
Mouse/Rat WISP-1 Conjugate	894361	12 mL of a polyclonal antibody specific for mouse/rat WISP-1 conjugated to horseradish peroxidase with preservatives.	May be stored for up to 1 month at 2-8 °C.*
Assay Diluent RD1-21	895215	12 mL of a buffered protein solution with preservatives.	
Calibrator Diluent RD6-31	895323	21 mL of diluted animal serum with preservatives. *Use diluted 1:2 in this assay.*	
Wash Buffer Concentrate	895003	21 mL of a 25-fold concentrated solution of buffered surfactant with preservative. *May turn yellow over time.*	
Color Reagent A	895000	12 mL of stabilized hydrogen peroxide.	
Color Reagent B	895001	12 mL of stabilized chromogen (tetramethylbenzidine).	
Stop Solution	895174	23 mL of diluted hydrochloric acid.	
Plate Sealers	N/A	4 adhesive strips.	

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

OTHER SUPPLIES REQUIRED

- Microplate reader capable of measuring absorbance at 450 nm, with the correction wavelength set at 540 nm or 570 nm.
- Pipettes and pipette tips.
- Deionized or distilled water.
- Squirt bottle, manifold dispenser, or automated microplate washer.
- 50 mL and 500 mL graduated cylinders.
- **Polypropylene** test tubes for dilution of standards and samples.

If using tissue lysate samples, the following are also required:

- Cell Lysis Buffer 2 (R&D Systems®, Catalog # 895347).
- PBS

PRECAUTIONS

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.

Color Reagent B may cause skin, eye, and respiratory irritation. Avoid breathing fumes.

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

SAMPLE COLLECTION & STORAGE

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Cell Culture Supernates - Remove particulates by centrifugation. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Tissue Lysates - Prior to assay, tissues must be lysed according to the directions in the Sample Values section.

Mouse Serum - Allow blood samples to clot for 2 hours at room temperature before centrifuging for 20 minutes at 2000 x g. Remove serum and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Rat Serum - Allow blood samples to clot for 2 hours at room temperature before centrifuging for 20 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Mouse Plasma - Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge for 20 minutes at 2000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Rat Plasma - Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge for 20 minutes at 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Note: *Citrate plasma has not been validated for use in this assay.*

Urine - Collect urine using a metabolic cage. Remove any particulates by centrifugation and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles. Centrifuge again before assaying to remove any additional precipitates that may appear after storage.

SAMPLE PREPARATION

Cell culture supernate and urine samples may require dilution.

Serum and plasma samples require a 4-fold dilution prior to assay. A suggested 4-fold dilution is 50 µL of sample + 150 µL of Calibrator Diluent RD6-31 (diluted 1:2)*.

*See Reagent Preparation section.

REAGENT PREPARATION

Bring all reagents to room temperature before use.

Mouse/Rat WISP-1 Control - Reconstitute the control with 1.0 mL of deionized or distilled water. Mix thoroughly. Assay the control undiluted.

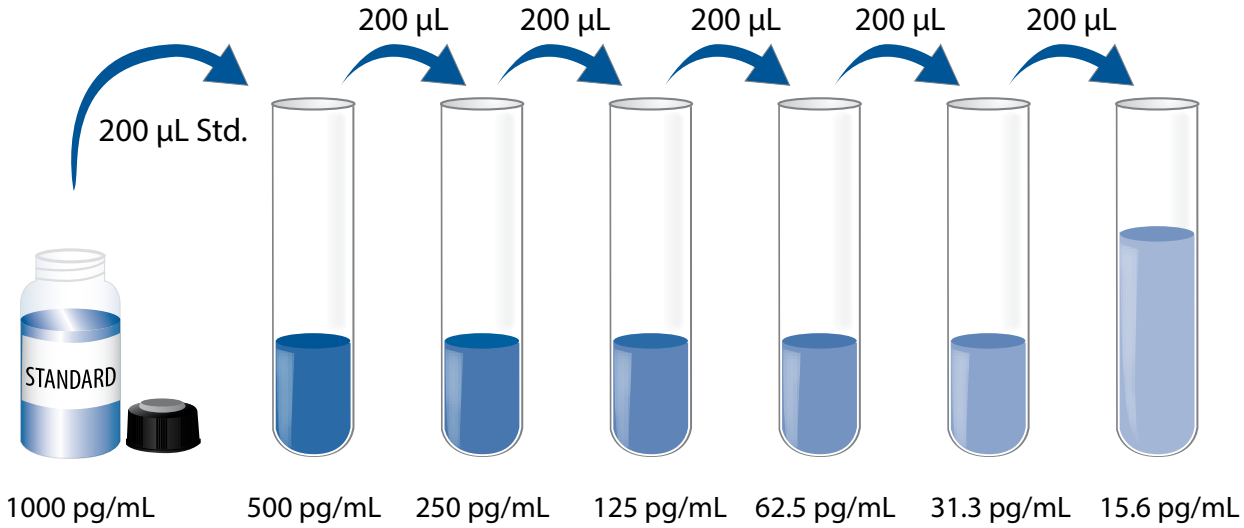
Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Add 20 mL of Wash Buffer Concentrate to 480 mL of deionized or distilled water to prepare 500 mL of Wash Buffer.

Substrate Solution - Color Reagents A and B should be mixed together in equal volumes within 15 minutes of use. Protect from light. 100 µL of the resultant mixture is required per well.

Calibrator Diluent RD6-31 (diluted 1:2) - Add 20 mL of Calibrator Diluent RD6-31 to 20 mL of deionized or distilled water to prepare 40 mL of Calibrator Diluent RD6-31 (diluted 1:2).

Mouse/Rat WISP-1 Standard - **Refer to the vial label for reconstitution volume.** Reconstitute the Mouse/Rat WISP-1 Standard with Calibrator Diluent RD6-31 (diluted 1:2). This reconstitution produces a stock solution of 1000 pg/mL. Allow the stock solution to sit for a minimum of 15 minutes with gentle mixing prior to making dilutions.

Use polypropylene tubes. Pipette 200 µL of Calibrator Diluent RD6-31 (diluted 1:2) into each tube. Use the stock solution to produce dilution series (below). Mix each tube thoroughly before the next transfer. The Mouse/Rat WISP-1 Standard (1000 pg/mL) serves as the high standard. Calibrator Diluent RD6-31 (diluted 1:2) serves as the zero standard (0 pg/mL).



ASSAY PROCEDURE

Bring all reagents and samples to room temperature before use. It is recommended that all standards, control, and samples be assayed in duplicate.

1. Prepare all reagents, standard dilutions, control, and samples as directed in the previous sections.
2. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal.
3. Add 50 µL of Assay Diluent RD1-21 to each well.
4. Add 50 µL of standard, control, or sample* per well. Gently tap the plate to ensure thorough mixing. Cover with the adhesive strip provided. Incubate for 2 hours at room temperature. A plate layout is provided to record standards and samples assayed.

   Note: *Pipette standards, control, and samples within 15 minutes.*
5. Aspirate each well and wash, repeating the process three times for a total of four 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 to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
6. Add 100 µL of Mouse/Rat WISP-1 Conjugate to each well. Cover with a new adhesive strip. Incubate for 2 hours at room temperature.
7. Repeat the aspiration/wash as in step 5.
8. Add 100 µL of Substrate Solution to each well. Incubate for 30 minutes at room temperature. **Protect from light.**
9. Add 100 µL of Stop Solution to each well. Gently tap the plate to ensure thorough mixing.
10. Determine the optical density of each well within 30 minutes, 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.

*Samples may require dilution. See the Sample Preparation section.

CALCULATION OF RESULTS

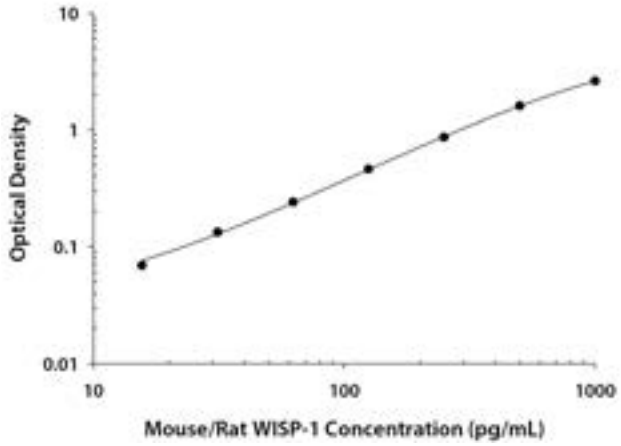
Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density (O.D.).

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 mouse/rat WISP-1 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.

If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

TYPICAL DATA

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



(pg/mL)	O.D.	Average	Corrected
0	0.031 0.032	0.032	—
15.6	0.097 0.103	0.100	0.068
31.3	0.161 0.167	0.164	0.132
62.5	0.272 0.273	0.273	0.241
125	0.474 0.513	0.494	0.462
250	0.886 0.910	0.898	0.866
500	1.588 1.675	1.632	1.600
1000	2.616 2.678	2.647	2.615

PRECISION

Intra-Assay Precision (Precision within an assay)
Three samples of known concentration were tested twenty times on one plate to assess intra-assay precision.

Inter-Assay Precision (Precision between assays)
Three samples of known concentration were tested in twenty separate assays to assess inter-assay precision. Assays were performed by at least three technicians using two lots of components.

	Intra-Assay Precision			Inter-Assay Precision		
Sample	1	2	3	1	2	3
n	20	20	20	20	20	20
Mean (pg/mL)	33.8	106	323	37.3	93.7	288
Standard deviation	1.58	4.06	15.9	3.59	6.16	11.1
CV (%)	4.7	3.8	4.9	9.6	6.6	3.9

RECOVERY

The recovery of mouse/rat WISP-1 spiked to levels throughout the range of the assay in various matrices was evaluated.

Mouse Samples	Average % Recovery	Range
Cell culture samples (n=4)	104	89-119%
Tissue lysates (n=4)	89	80-111%
Serum* (n=4)	104	90-119%
EDTA plasma* (n=4)	110	96-119%
Heparin plasma* (n=4)	104	81-120%
Urine* (n=4)	107	90-120%

*Samples were diluted prior to assay as directed in the Sample Preparation section.
Note: *Rat samples were evaluated and no significant difference in recovery was observed from the data above.*

SENSITIVITY

Thirty-four assays were evaluated and the minimum detectable dose (MDD) of mouse/rat WISP-1 ranged from 0.514-2.74 pg/mL. The mean MDD was 1.42 pg/mL.

The MDD was determined by adding two standard deviations to the mean O.D. value of twenty zero standard replicates and calculating the corresponding concentration.

LINEARITY

To assess the linearity of the assay, samples containing high concentrations of mouse/rat WISP-1 in each matrix were diluted with calibrator diluent and assayed.

Mouse Samples		Cell culture samples* (n=4)	Tissue lysates (n=4)	Serum* (n=4)	EDTA plasma* (n=4)	Heparin plasma* (n=4)	Urine* (n=4)
1:2	Average % of Expected	100	100	103	107	103	104
	Range (%)	95-107	98-102	97-111	105-111	92-109	96-108
1:4	Average % of Expected	103	105	104	109	101	103
	Range (%)	91-114	102-108	99-109	106-118	92-113	92-113
1:8	Average % of Expected	98	112	109	113	101	102
	Range (%)	91-110	101-118	105-115	105-119	94-114	88-114
1:16	Average % of Expected	97	115	108	112	105	99
	Range (%)	92-104	114-116	91-117	103-116	98-120	90-107

*Samples were diluted prior to assay.

Note: *Rat samples were evaluated and no significant difference in linearity was observed from the data above.*

CALIBRATION

This immunoassay is calibrated against a highly purified NS0-expressed recombinant mouse WISP-1 produced at R&D Systems®.

SAMPLE VALUES

Serum/Plasma/Urine - Samples were evaluated for the presence of mouse/rat WISP-1 in this assay.

Mouse Samples	Mean (pg/mL)	Range (pg/mL)	Standard Deviation (pg/mL)
Serum (n=10)	988	527-1485	310
EDTA plasma (n=5)	817	766-962	82
Heparin plasma (n=5)	886	794-1044	96

Rat Samples	Mean (pg/mL)	Range (pg/mL)	Standard Deviation (pg/mL)
Serum (n=10)	1155	482-2409	628
EDTA plasma (n=5)	1747	892-3348	1053
Heparin plasma (n=5)	1354	1209-1507	134

	Mean of Detectable (pg/mL)	% Detectable	Range (pg/mL)
Mouse urine (n=10)	11,889	90	ND-31,516
Rat urine (n=10)	424	80	ND-856

ND=Non-detectable

SAMPLES VALUES *CONTINUED*

Cell Culture Supernates:
3T3-L1 mouse embryonic fibroblast adipose-like cells were cultured in DMEM supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin sulfate for 3 days. Cell culture supernate was removed and fresh media was added. For differentiation, 1.0 µg/mL bovine insulin, 0.5 mM MIX, and 1 µM DEX were added to the media. Cells were cultured for 4 days. Cell culture supernate was removed and fresh media was added. Cells were cultured an additional 4 days for a total of 11 days. Aliquots of the cell culture supernates were removed and assayed for levels of mouse WISP-1.

	Day 7 (pg/mL)	Day 11 (pg/mL)
Undifferentiated	912	844
Differentiated	239	186

C2C12 mouse myoblast cells were cultured in DMEM supplemented with 10% fetal bovine serum or C2C12 cells differentiated into muscle cells were cultured with DMEM supplemented with 2% equine serum for 6 days. Aliquots of the cell culture supernates were removed and assayed for levels of mouse WISP-1.

	(pg/mL)
Undifferentiated	4813
Differentiated	6740

L6 rat myoblast cells were cultured in DMEM supplemented with 10% fetal bovine serum and 1 mM sodium pyruvate for 4 days. An aliquot of the cell culture supernate was removed, assayed for rat WISP-1, and measured 1034 pg/mL.

Tissue Lysates - Organs from mice were rinsed with PBS and homogenized with a tissue homogenizer in PBS. An equal volume of Cell Lysis Buffer 2 was added and tissues were lysed at room temperature for 30 minutes with gentle agitation. Debris was then removed by centrifugation. Aliquots of the lysates were removed and assayed for levels of mouse WISP-1.

Mouse Lysates	(pg/mL)
Brain	132
Heart	46.1
Kidney	462
Liver	203
Lung	54.5
Spleen	379

SPECIFICITY

This assay recognizes natural and recombinant mouse/rat WISP-1.

The factors listed below were prepared at 10 ng/mL in calibrator diluent and assayed for cross-reactivity. Preparations of the following factors at 10 ng/mL in a mid-range mouse/rat WISP-1 control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant mouse:

Cyr61
Decorin
IGFBP-1
IGFBP-2
IGFBP-3
IGFBP-4
IGFBP-5
IGFBP-6
IGFBP-7
IGFBP-L1
IGFBP-rP10
Integrin α5
Integrin β1
NOV/CCN3
R-spondin 1
R-spondin 2
R-spondin 3
R-spondin 4
THSD1

Recombinant human:

Biglycan
BMP-2
CTGF

Other factors:

Heparin (2% v/v)

Recombinant human WISP-1 does not interfere but does cross-react approximately 7% in this assay.

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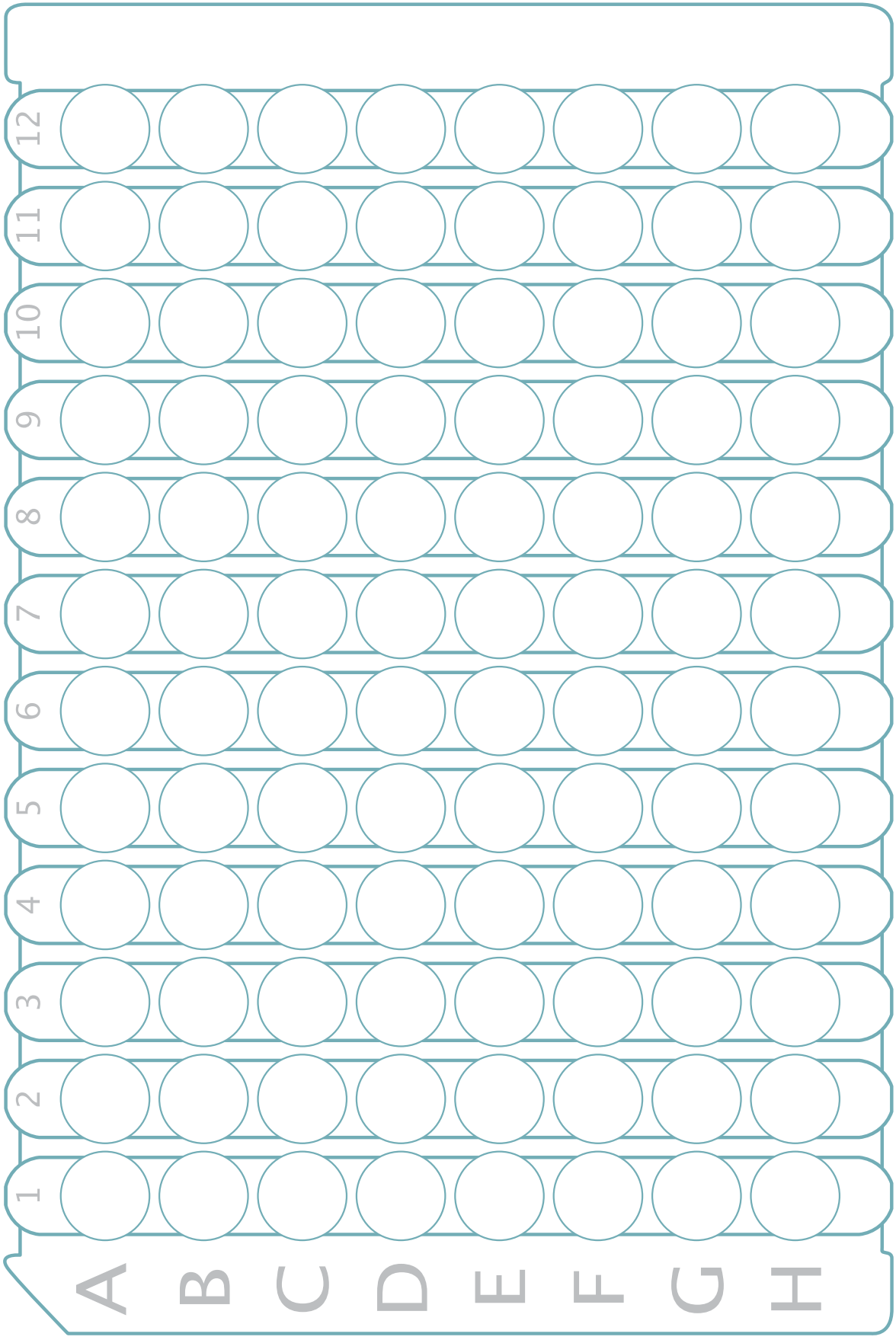
PLATE LAYOUT

Use this plate layout to record standards and samples assayed.



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