Quantikine™ ELISA

Human Pro-MMP-1 Immunoassay

Catalog Number DMP100
SMP100
PDMP100

For the quantitative determination of human Pro-Matrix Metalloproteinase 1 (Pro-MMP-1) concentrations in cell culture supernates, serum, and plasma.

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

Matrix metalloproteinases (MMPs) play an important role in physiological and pathological processes including embryogenesis, tissue remodeling, wound healing, inflammation, arthritis and cancer. They are a family of zinc and calcium dependent endopeptidases that function in the turnover of components of the extracellular matrix. They are secreted as zymogens (Pro-MMPs) that are activated by a variety of proteinases or by reaction with organic mercurials. They are inhibited by proteins including specific tissue inhibitors of metalloproteinases (TIMPs), by α_2-macroglobulin and by metal chelators such as phenanthroline and EDTA (1-4).

MMP-1 (also referred to as interstitial collagenase, vertebrate collagenase, fibroblast collagenase, and collagenase I) is produced by fibroblasts, chondrocytes, macrophages, keratinocytes, endothelial cells and osteoblasts. The production of MMP-1 is upregulated by a variety of stimuli including cytokines such as EGF, interleukins and TNF-α, chemical agents such as cAMP and phorbol esters, and events occurring at the cell surface such as cell fusion and phagocytosis. MMP-1 is inhibited stoichiometrically by TIMP-1 and TIMP-2 and is rapidly inhibited by α_2-macroglobulin. Structurally, the 450 amino acid sequence of MMP-1 consists of an N-terminal pro domain (80 residues) that is cleaved upon activation, a catalytic domain (162 residues) containing the zinc-binding site, a linking peptide (16 residues) and a C-terminal hemopexin-like domain (192 residues) (5, 6).

MMP-1 plays a significant role in the degradation of fibrillar collagens in extracellular matrix remodeling, characterized by the cleavage of the interstitial collagen triple helix into ¾ and ¼ fragments. MMP-1 is therefore implicated in a wide variety of biological processes where collagen degradation occurs. These include rheumatoid arthritis, osteoarthritis, periodontal disease, tumor invasion, angiogenesis, corneal ulceration, tissue remodeling, inflammatory bowel disease, atherosclerosis, aneurysm, and restenosis (1-4). In addition, MMP-1 can also cleave a variety of other substrates such as casein, gelatin, aggrecan, entactin, Pro-TNF, and cartilage link protein (5, 6). Thus, the role of MMP-1 is more diverse than originally believed, and may involve enzyme cascades, cytokine regulation and cell surface modulation.

The Quantikine™ Human Pro-MMP-1 Immunoassay is a 4.5 hour solid phase ELISA designed to measure human Pro-MMP-1 in cell culture supernates, serum, and plasma. It contains NS0-expressed recombinant human Pro-MMP-1 and antibodies raised against the recombinant factor. The antibodies will not recognize recombinant human active MMP-1 or TIMP bound MMP-1. Natural human Pro-MMP-1 showed dose-response curves that were parallel to the standard curves obtained using the recombinant Quantikine kit standards. These results indicate that this kit can be used to determine relative mass values of natural human Pro-MMP-1.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for human Pro-MMP-1 has been pre-coated onto a microplate. Standards and samples are pipetted into the wells, and Pro-MMP-1 is bound by the immobilized antibody. After washing away unbound substances, an enzyme-linked monoclonal antibody specific for human Pro-MMP-1 is added to the wells. Following a wash to remove unbound antibody-enzyme reagent, a substrate solution is added to the wells and color develops in proportion to the amount of Pro-MMP-1 bound in the initial step. The color development is stopped and the intensity of the color is measured.

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.
- If samples generate values higher than the highest standard, dilute the samples with the appropriate 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.
- When using an automated plate washer, adding a 30 second soak period following the addition of Wash Buffer, and/or rotating the plate 180 degrees between wash steps may improve assay precision.
- 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. Wells that are green in color indicate that the Stop Solution has not mixed thoroughly with the Substrate Solution.

MATERIALS PROVIDED & STORAGE CONDITIONS

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

PART	PART #	CATALOG # DMP100	CATALOG # SMP100	DESCRIPTION	STORAGE OF OPENED/ RECONSTITUTED MATERIAL
Human Pro-MMP-1 Microplate	890754	1 plate	6 plates	96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody specific for human Pro-MMP-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.*
Human Pro-MMP-1 Standard	890756	2 vials	12 vials	Recombinant human Pro-MMP-1 in a buffered protein base with preservatives; lyophilized. *Refer to the vial label for reconstitution volume.*	Use a new standard for each assay. Discard after use.
Human Pro-MMP-1 Conjugate	890755	1 vial	6 vials	21 mL/vial of a monoclonal antibody specific for human Pro-MMP-1 conjugated to horseradish peroxidase with preservatives.	May be stored for up to 1 month at 2-8 °C.*
Assay Diluent RD1-52	895343	1 vial	6 vials	11 mL/vial of a buffered protein base with blue dye and preservatives. *May contain a precipitate. Mix well before and during use.*	
Calibrator Diluent RD5-19	895344	1 vial	6 vials	21 mL/vial of a buffered protein base with preservatives. *For cell culture supernate samples.*	
Calibrator Diluent RD6-28	895345	1 vial	6 vials	21 mL/vial of diluted animal serum with preservatives. *For serum/plasma samples.*	
Wash Buffer Concentrate	895003	1 vial	6 vials	21 mL/vial of a 25-fold concentrated solution of buffered surfactant with preservative. *May turn yellow over time.*	
Color Reagent A	895000	1 vial	6 vials	12 mL/vial of stabilized hydrogen peroxide.	
Color Reagent B	895001	1 vial	6 vials	12 mL/vial of stabilized chromogen (tetramethylbenzidine).	
Stop Solution	895032	1 vial	6 vials	6 mL/vial of 2 N sulfuric acid.	
Plate Sealers	N/A	4 strips	24 strips	Adhesive strips.	

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

DMP100 contains sufficient materials to run an ELISA on one 96 well plate.
SMP100 (SixPak) contains sufficient materials to run ELISAs on six 96 well plates.

This kit is also available in a PharmPak (R&D Systems®, Catalog # PDMP100). Refer to the literature accompanying your order for specific vial counts.

PHARMPAK CONTENTS

Each PharmPak contains reagents sufficient for the assay of 50 microplates (96 wells/plate). The package inserts supplied are the same as those supplied in the single kit packs and because of this, a few minor differences related to the number of reagents and their container sizes should be noted.

- Sufficient material is supplied to perform at least 50 standard curves; reuse of each vial may be required. The number of vials, and the number of standard curves obtained per vial will vary with the analyte.
- Wash Buffer 25X Concentrate is bulk packed in 125 mL bottles containing 100 mL.
  Note: *Additional wash buffer is available for purchase (R&D Systems®, Catalog # WA126).*

The reagents provided in this PharmPak are detailed below.

PART	PART #	QUANTITY
Human Pro-MMP-1 Microplate	890754	50 plates
Human Pro-MMP-1 Conjugate	890755	50 vials
Human Pro-MMP-1 Standard	890756	50 vials
Calibrator Diluent RD5-19	895344	50 vials
or		
Calibrator Diluent RD6-28	895345	50 vials
Assay Diluent RD1-52	895343	50 vials
Color Reagent A	895000	50 vials
Color Reagent B	895001	50 vials
Wash Buffer Concentrate	895126	9 bottles
Stop Solution	895032	50 vials
Plate sealers	N/A	100 sheets
Package inserts	750454	2 booklets

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
- 500 mL graduated cylinder
- Deionized or distilled water
- Squirt bottle, manifold dispenser, or automated microplate washer
- Horizontal orbital microplate shaker (0.12" orbit) capable of maintaining a speed of 500 ± 50 rpm
- Test tubes for dilution of standards
- Human Pro-MMP-1 controls (optional; R&D Systems®, Catalog #QC125)

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 and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Note: *RPMI is not recommended for use in this assay. RPMI contains phosphate, which causes Pro-MMP-1 instability.*

Serum - Use a serum separator tube (SST) and allow samples to clot for 30 minutes at room temperature before centrifugation for 15 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Plasma - Collect plasma using heparin as an anticoagulant. Centrifuge for 15 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: *EDTA and citrate plasma are not recommended for use in this assay due to their chelating properties.*

REAGENT PREPARATION

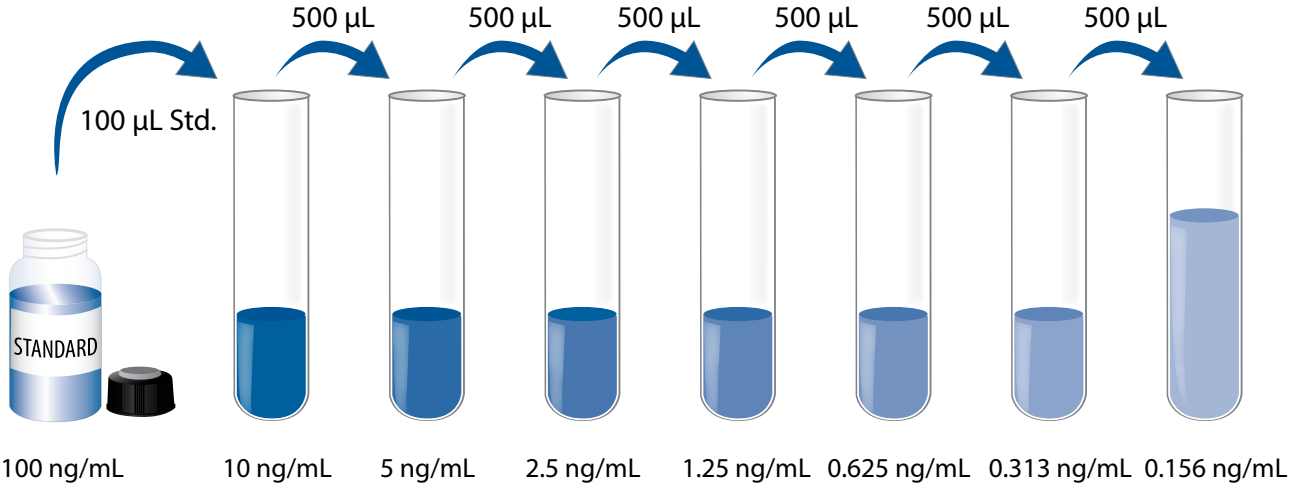
Bring all reagents to room temperature before use.

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. 200 µL of the resultant mixture is required per well.

Human Pro-MMP-1 Standard - **Refer to the vial label for reconstitution volume.** Reconstitute the Human Pro-MMP-1 Standard with deionized or distilled water. This reconstitution produces a stock solution of 100 ng/mL. Allow the standard to sit for **30-90 minutes** with gentle agitation prior to making dilutions.

Pipette 900 µL of Calibrator Diluent RD5-19 (*for cell culture supernate samples*) or Calibrator Diluent RD6-28 (*for serum/plasma samples*) into the 10 ng/mL tube. Pipette 500 µL of the appropriate calibrator diluent into the remaining tubes. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The 10 ng/mL standard serves as the high standard. The appropriate calibrator diluent serves as the zero standard (0 ng/mL). **Use diluted standards within 60 minutes of preparation.**



ASSAY PROCEDURE

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

1. Prepare all reagents, working standards, 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 100 µL of Assay Diluent RD1-52 to each well. *Assay Diluent RD1-52 may contain a precipitate. Mix well before and during use.*
4. Add 100 µL of standard, control, or sample per well. Cover with the adhesive strip provided. Incubate for 2 hours at room temperature on a horizontal orbital microplate shaker (0.12" orbit) set at 500 ± 50 rpm.
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 200 µL of Human Pro-MMP-1 Conjugate to each well. Cover with a new adhesive strip. Incubate for 2 hours at room temperature on the shaker.
7. Repeat the aspiration/wash as in step 5.
8. Add 200 µL of Substrate Solution to each well. **Protect from light.**
   For cell culture supernate samples: Incubate for 20 minutes at room temperature **on the benchtop.**
   For serum/plasma samples: Incubate for 30 minutes at room temperature **on the benchtop.**
9. Add 50 µL of Stop Solution to each well. The color in the wells should change from blue to yellow. If the color in the wells is green or the color change does not appear uniform, 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.

CALCULATION OF RESULTS

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

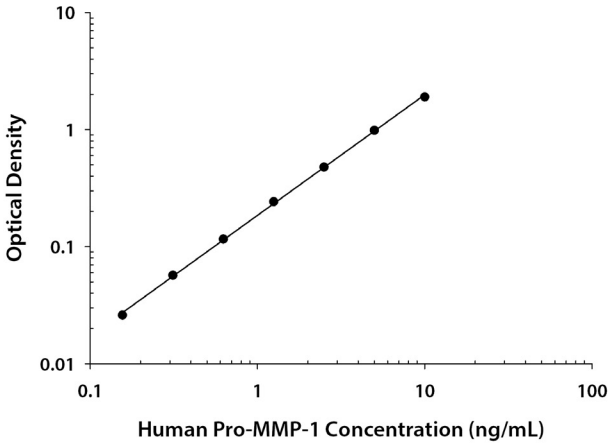
Create a standard curve by reducing the data using computer software capable of generating a log/log curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on a log/log graph. The data may be linearized by plotting the log of the human Pro-MMP-1 concentrations versus the log of the O.D. on a linear scale, and the best fit line can be determined by regression analysis.

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

TYPICAL DATA

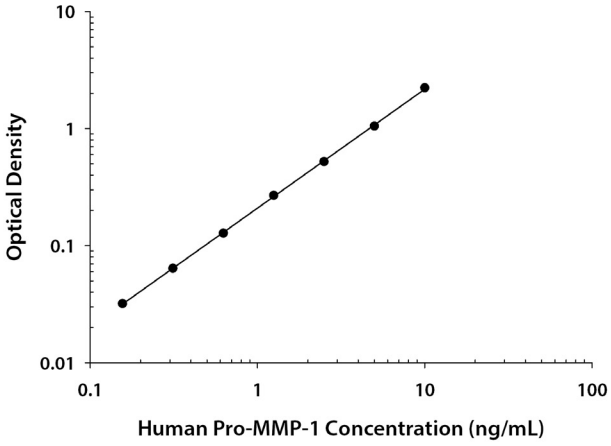
These standard curves are provided for demonstration only. A standard curve should be generated for each set of samples assayed.

CELL CULTURE SUPERNATE ASSAY



(ng/mL)	O.D.	Average	Corrected
0	0.030 0.028	0.029	—
0.156	0.055 0.055	0.055	0.026
0.313	0.086 0.086	0.086	0.057
0.625	0.147 0.143	0.145	0.116
1.25	0.283 0.259	0.271	0.242
2.5	0.512 0.502	0.507	0.478
5	1.027 1.001	1.014	0.985
10	1.948 1.911	1.930	1.901

SERUM/PLASMA ASSAY



(ng/mL)	O.D.	Average	Corrected
0	0.028 0.027	0.028	—
0.156	0.061 0.059	0.060	0.032
0.313	0.094 0.089	0.092	0.064
0.625	0.163 0.148	0.156	0.128
1.25	0.310 0.284	0.297	0.269
2.5	0.554 0.545	0.550	0.522
5	1.120 1.036	1.078	1.050
10	2.296 2.219	2.258	2.230

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 forty separate assays to assess inter-assay precision. Assays were performed by at least three technicians using two lots of components.

CELL CULTURE SUPERNATE ASSAY

	Intra-Assay Precision			Inter-Assay Precision		
Sample	1	2	3	1	2	3
n	20	20	20	40	40	40
Mean (ng/mL)	0.494	1.65	3.39	0.561	1.73	3.50
Standard deviation	0.022	0.065	0.177	0.065	0.153	0.270
CV (%)	4.5	3.9	5.2	11.6	8.8	7.7

SERUM/PLASMA ASSAY

	Intra-Assay Precision			Inter-Assay Precision		
Sample	1	2	3	1	2	3
n	20	20	20	40	40	40
Mean (ng/mL)	0.702	2.13	4.43	0.738	2.34	4.66
Standard deviation	0.039	0.130	0.241	0.077	0.176	0.312
CV (%)	5.6	6.1	5.4	10.4	7.5	6.7

RECOVERY

The recovery of human Pro-MMP-1 spiked to levels throughout the range of the assay in various matrices was evaluated.

Sample Type	Average % Recovery	Range
Cell culture media (n=4)	103	98-112%
Serum (n=5)	97	87-112%
Heparin plasma (n=5)	92	85-102%

SENSITIVITY

Fifty-seven assays were evaluated and the minimum detectable dose (MDD) of human Pro-MMP-1 ranged from 0.006-0.095 ng/mL. The mean MDD was 0.021 ng/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 and/or spiked with high concentrations of human Pro-MMP-1 in various matrices were diluted with calibrator diluent to produce samples with values within the dynamic range of the assay.

		Cell culture media (n=4)	Serum (n=8)	Heparin plasma (n=5)
1:2	Average % of Expected	102	108	108
	Range (%)	98-104	99-114	101-112
1:4	Average % of Expected	101	107	107
	Range (%)	92-105	95-113	96-113
1:8	Average % of Expected	99	99	106
	Range (%)	93-107	87-112	89-114
1:16	Average % of Expected	99	93	106
	Range (%)	88-106	86-100	90-113

CALIBRATION

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

SAMPLE VALUES

Serum/Plasma - Samples from apparently healthy volunteers were evaluated for the presence of human Pro-MMP-1 in this assay. No medical histories were available for the donors used in this study.

Sample Type	Mean (ng/mL)	Range (ng/mL)
Serum (n=60)	3.45	0.912-9.34
Heparin plasma (n=35)	0.475	0.179-1.00

Pro-MMP-1 is released during clotting; therefore, serum samples will contain higher levels of Pro-MMP-1.

SPECIFICITY

This assay recognizes natural and recombinant human Pro-MMP-1. The antibodies in this kit do not recognize recombinant human active MMP-1 or TIMP bound MMP-1.

The factors listed below were prepared at 200 ng/mL in calibrator diluent and assayed for cross-reactivity. Preparations of the same factors prepared at 200 ng/mL in a mid-range recombinant human Pro-MMP-1 Standard were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant human:

MMP-2
MMP-3
MMP-9
TIMP-1
TIMP-2

REFERENCES

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2. Nagase, H. (1996) "Matrix metalloproteinases" in *Zinc Metalloproteases in Health and Disease,* Hooper, N.M., ed., Taylor and Francis, Bristol, PA, pp. 153-204.
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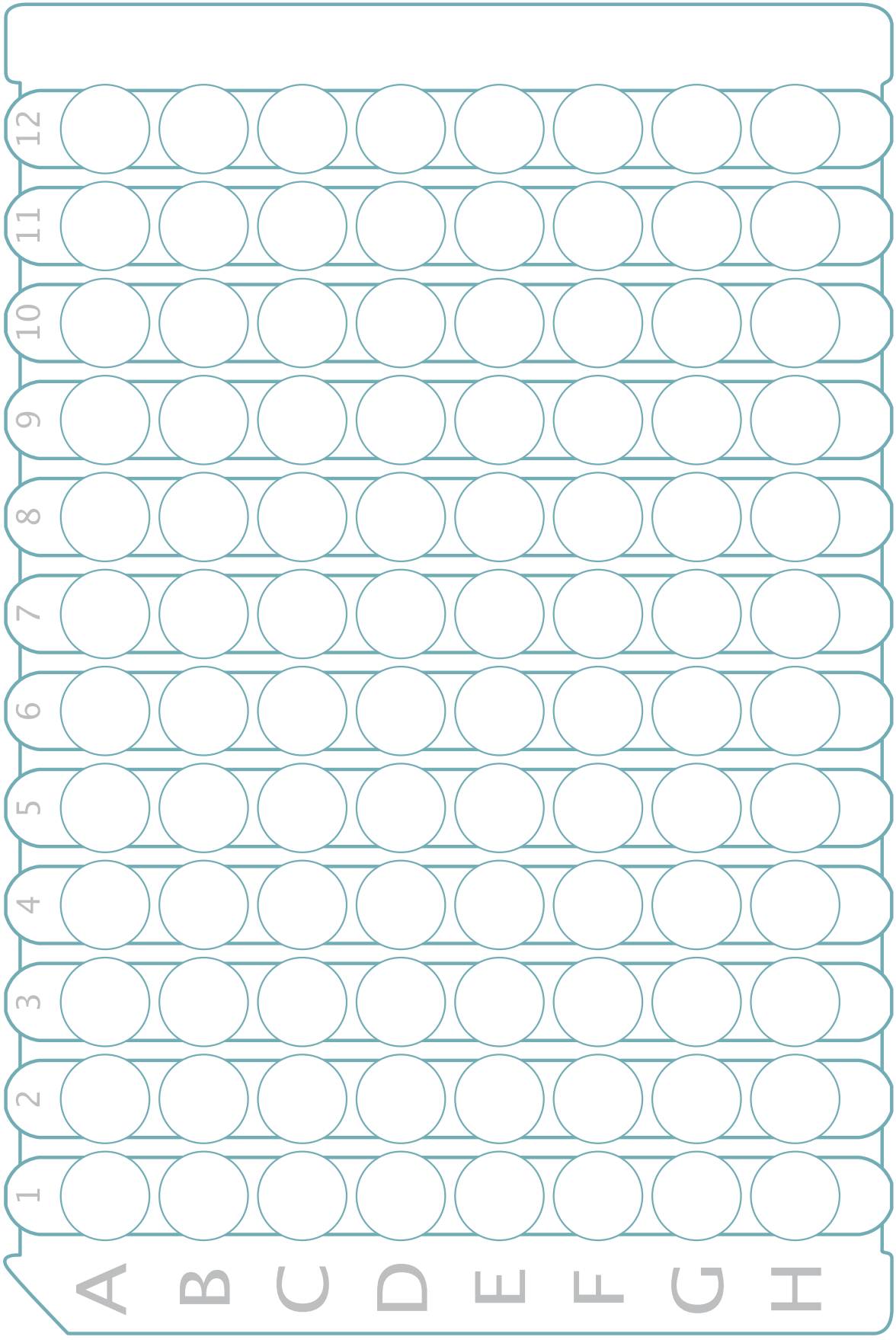
4. Woessner, J.F. Jr. (1991) FASEB J. **5**:2145.
5. Cawston, T.E. (1998) "Interstitial Collagenases" in *Handbook of Proteolytic Enzymes*, Barrett, A.J., Rawlings, N.D. and Woessner, J.F. eds., Academic Press, San Diego, CA, pp. 1155-1162.
6. Dioszegi, M. *et al.* (1995) Methods Enzymol. **248**:413.

PLATE LAYOUT

Use this plate layout to record standards and samples assayed.



NOTES