Quantikine® ELISA

Human IL-12 p70 Immunoassay

Catalog Number D1200
S1200
PD1200

For the quantitative determination of human Interleukin 12 p70 (IL-12 p70) concentrations in cell culture supernates, serum, plasma, and urine.

Note: The standard reconstitution method has changed. Please read this package insert in its entirety before using this product.

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

Interleukin 12 (IL-12), also known as NKSF, is a 70-75 kDa heterodimeric glycoprotein that belongs to the IL-12 family of heterodimeric cytokines (1-3). It consists of two disulfide-linked subunits which are 35 kDa (p35) and 40 kDa (p40) in size and share no meaningful amino acid (aa) sequence identity with each other (1, 4, 5). The mature p35 subunit is 197 aa in length and contains seven cysteines plus one potential N-linked glycosylation site (1-6). Mature human p35 shares 58% aa identity with mouse and rat p35 (2, 7-9). Mature human p40 is 306 aa in length, with 11 cysteines and three potential N-linked glycosylation sites, and it shares 66% aa identity with mouse and rat p40, respectively (1, 7, 10). While p35 resembles a hematopoietin ligand, p40 strongly resembles the N-terminus of a hematopoietin receptor, exhibiting a WSxWS motif, an immunoglobulin-like domain, and four conserved cysteines (1). This suggests that IL-12 may be a cytokine-receptor analog to the IL-6/soluble IL-6 R complex (4, 6). Notably, while p40 may circulate as either a monomer or homodimer, p35 is never found by itself (3). p40 does, however, serve as the larger of two subunits that comprise IL-23 (3, 11). Finally, while IL-12 is classically thought of as a secreted molecule, membrane-bound IL-12 has been reported on both human and mouse cells (12). Cells known to produce IL-12 include macrophages and dendritic cells (13), monocytes (14), Langerhans cells (15), neutrophils (16), keratinocytes (17), plasmacytoid dendritic cells (18), microglia (5), $CD8^+$ DC (mouse cells only) (19) and non-germinal center ($CD38^-CD44^+$) B cells (human cells only) (3, 20).

The high affinity receptor for human IL-12 is composed of at least two type I transmembrane glycoproteins that resemble members of the cytokine receptor superfamily. The first subunit (Rβ1) is 100 kDa in size and binds IL-12 with a Kd=1 nM (21). This receptor serves as the principal binding site for the p40 subunit (4, 5). The second subunit (Rβ2) is 130 kDa in size and shows no meaningful aa sequence identity to the Rβ1 subunit (5, 21, 22). This receptor appears to be the principal signal transduction component and is suggested to serve as an attachment point for a disulfide-linked p35-p40 heterodimer (4, 5, 22). As noted above, human p40 will circulate either as a monomer, homodimer, or in a complex bound to either p35, forming IL-12, or to p19, forming IL-23 (3-5, 11). Both the homodimeric p40, and IL-23 can bind to the IL-12 R, serving as nonsignaling antagonists (3, 23, 24). Alternatively, the p40 homodimer may also bind to Rβ1, activating microglia and macrophages (4, 25).

Functionally, IL-12 has been shown to both enhance cytotoxic activity and induce interferon-gamma (IFN-γ) production in NK cells, T cells and dendritic epidermal T cells (3, 26-28). IL-12 has also been reported to induce IFN-γ production in macrophages (29). IL-12, in conjunction with the other IL-12 family members IL-23 and IL-27, promotes the development of a $CD4^+$ Th1 immune response (4, 5, 30). In response to infection, IL-27 is released initially, promoting a Th0 to Th0/1 transition. IL-12 production follows, generating Th1 effector cells. In combination with IL-18, IL-12 creates Th1 memory cells out of effector cells, and these cells are later activated by IL-23 (4).

The Quantikine® Human IL-12 p70 Immunoassay is a 3.5-4.5 hour solid phase ELISA designed to measure IL-12 p70 in cell culture supernates, serum, plasma, and urine. It contains *Sf* 21-expressed recombinant human IL-12 p70 and antibodies raised against the recombinant factor. This immunoassay has been shown to quantitate recombinant human IL-12 p70 accurately. Results obtained using natural human IL-12 p70 showed linear curves that were parallel to the standard curves obtained using the recombinant kit standards. These results indicate that this kit can be used to determine relative mass values for natural human IL-12 p70.

This kit was developed with a capture antibody that recognizes only the IL-12 p70 heterodimer and not the individual subunits of the dimer, thus eliminating the potential for interference by these subunits.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for human IL-12 p70 has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any IL-12 p70 present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for human IL-12 p70 is added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, a substrate solution is added to the wells and color develops in proportion to the amount of IL-12 p70 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 Calibrator Diluent and repeat the assay.
- Any variation in standard 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 # D1200	CATALOG # S1200	DESCRIPTION	STORAGE OF OPENED/ RECONSTITUTED MATERIAL
Human IL-12 p70 Microplate	890212	1 plate	6 plates	96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody specific for human IL-12 p70.	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 IL-12 p70 Standard	890214	1 vial	6 vials	Recombinant human IL-12 p70 in a buffered protein base with preservatives; lyophilized. *Refer to the vial label for reconstitution volume.*	Aliquot and store for up to 1 month at 2-8 °C.*
Human IL-12 p70 Conjugate	890213	1 vial	6 vials	21 mL/vial of polyclonal antibody specific for human IL-12 p70 conjugated to horseradish peroxidase with preservatives.	May be stored for up to 1 month at 2-8 °C.*
Assay Diluent RD1F	895041	1 vial	6 vials	6 mL/vial of a buffered protein base with preservatives. *For cell culture supernate/serum/plasma samples.*	
Assay Diluent RD1U	895138	1 vial	6 vials	6 mL/vial of a buffered protein base with preservatives. *For urine samples. Contains a precipitate. Mix well before and during use.*	
Calibrator Diluent RD5C Concentrate	895046	1 vial	6 vials	21 mL/vial of a concentrated buffered protein base with preservatives. *Use diluted 1:5 in this assay.*	
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.

D1200 contains sufficient materials to run an ELISA on one 96 well plate.
S1200 (SixPak) contains sufficient materials to run ELISAs on six 96 well plates.

This kit is also available in a PharmPak (R&D Systems®, Catalog # PD1200). PharmPaks contain sufficient materials to run ELISAs on 50 microplates. Specific vial counts of each component may vary. Please refer to the literature accompanying your order for specific vial counts.

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.
- 100 mL and 500 mL graduated cylinders.
- Deionized or distilled water.
- Squirt bottle, manifold dispenser, or automated microplate washer.
- Test tubes for dilution of standards.
- Human IL-12 p70 Controls (optional; R&D Systems®, Catalog # QC01-1).

PRECAUTIONS

Calibrator Diluent RD5C and Human IL-12 p70 Standard contain sodium azide which may react with lead and copper plumbing to form explosive metallic azides. Flush with large volumes of water during disposal.

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 MSDS 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.

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 EDTA, heparin, or citrate 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.

Urine - Aseptically collect the first urine of the day (mid-stream), voided directly into a sterile container. Centrifuge to remove particulate matter. Assay immediately or aliquot and store at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

REAGENT PREPARATION

Bring all reagents to room temperature before use.

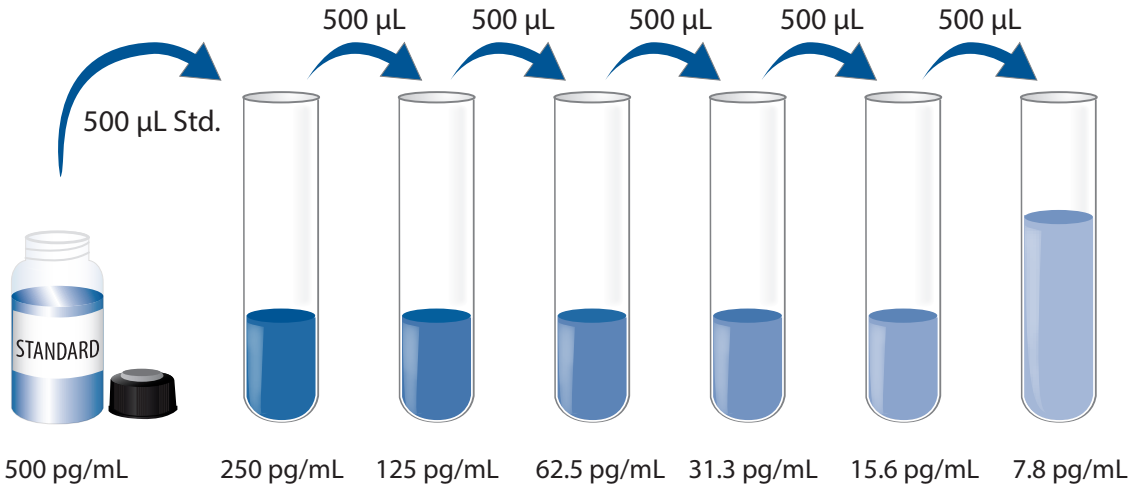
Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Add 20 mL of Wash Buffer Concentrate to deionized or distilled water to prepare 500 mL of Wash Buffer.

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.

Calibrator Diluent RD5C (diluted 1:5) - Add 20 mL of Calibrator Diluent RD5C Concentrate to 80 mL of deionized or distilled water to prepare 100 mL of Calibrator Diluent RD5C (diluted 1:5).

Human IL-12 p70 Standard - **Refer to the vial label for reconstitution volume.** Reconstitute the Human IL-12 p70 Standard with Calibrator Diluent RD5C (diluted 1:5). This reconstitution produces a stock solution of 500 pg/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions.

Pipette 500 µL of Calibrator Diluent RD5C (diluted 1:5) into each tube. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The undiluted Human IL-12 p70 Standard (500 pg/mL) serves as the high standard. Calibrator Diluent RD5C (diluted 1:5) 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, samples, and controls 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 50 µL of assay diluent to each well.
   For Cell Culture Supernate/Serum/Plasma Samples: Add Assay Diluent RD1F.
   For Urine Samples: Add Assay Diluent RD1U. *Assay Diluent RD1U contains a precipitate. Mix well before and during use.*
4. Add 200 µL of standard, control, or sample per well. Cover with the adhesive strip provided. A plate layout is provided to record standards and samples assayed.
   For Cell Culture Supernate/Serum/Plasma Samples: Incubate for 2 hours at room temperature.
   For Urine Samples: Incubate for 1.5 hours at room temperature.
5. Aspirate each well and wash, repeating the process twice for a total of three 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 toweling.
6. Add 200 µL of Human IL-12 p70 Conjugate to each well. Cover with a new adhesive strip.
   For Cell Culture Supernate/Serum/Plasma Samples: Incubate for 2 hours at room temperature.
   For Urine Samples: Incubate for 1.5 hours at room temperature.
7. Repeat the aspiration/wash as in step 5.
8. Add 200 µL of Substrate Solution to each well. Incubate for 20 minutes at room temperature. **Protect from light.**
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.).

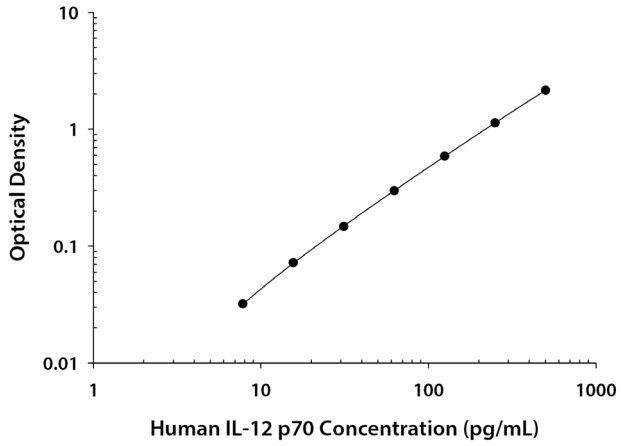
Create a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the log of the human IL-12 p70 concentration 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

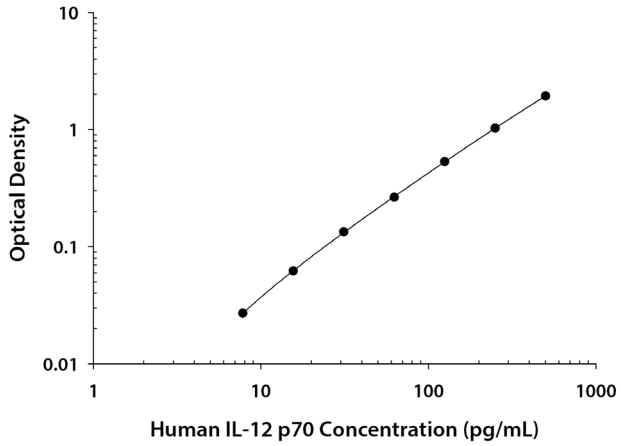
These standard curves are provided for demonstration only. A standard curve should be generated for each set of samples assayed.

CELL CULTURE SUPERNATE/SERUM/PLASMA ASSAY



(pg/mL)	O.D.	Average	Corrected
0	0.036 0.036	0.036	—
7.8	0.067 0.069	0.068	0.032
15.6	0.107 0.109	0.108	0.072
31.3	0.182 0.184	0.183	0.147
62.5	0.333 0.335	0.334	0.298
125	0.619 0.628	0.624	0.588
250	1.147 1.193	1.170	1.134
500	2.171 2.207	2.189	2.153

URINE ASSAY



(pg/mL)	O.D.	Average	Corrected
0	0.060 0.064	0.062	—
7.8	0.089 0.089	0.089	0.027
15.6	0.121 0.126	0.124	0.062
31.3	0.192 0.201	0.196	0.134
62.5	0.321 0.332	0.326	0.264
125	0.587 0.603	0.595	0.533
250	1.058 1.117	1.088	1.026
500	1.978 2.013	1.996	1.934

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.

CELL CULTURE SUPERNATE/SERUM/PLASMA ASSAY

	Intra-Assay Precision			Inter-Assay Precision		
Sample	**1**	**2**	**3**	**1**	**2**	**3**
n	20	20	20	20	20	20
Mean (pg/mL)	21.2	228	447	21.0	126	246
Standard deviation	0.3	3.5	4.9	1.5	5.6	8.1
CV (%)	1.4	1.5	1.1	7.1	4.4	3.3

URINE ASSAY

	Intra-Assay Precision			Inter-Assay Precision		
Sample	**1**	**2**	**3**	**1**	**2**	**3**
n	20	20	20	20	20	20
Mean (pg/mL)	25.7	202	436	19.9	115	223
Standard deviation	1.6	4.8	10.1	1.9	8.3	13.2
CV (%)	6.2	2.4	2.3	9.5	7.2	5.9

RECOVERY

The recovery of human IL-12 p70 spiked to three levels throughout the range of the assay in various matrices was evaluated.

Sample Type	Average % Recovery	Range
Cell culture media (n=5)	90	81- 96%
Serum (n=5)	100	89-106%
EDTA plasma (n=5)	101	96-109%
Heparin plasma (n=5)	99	89-109%
Citrate plasma (n=5)	90	82- 94%
Urine (n=5)	89	85- 92%

LINEARITY

To assess the linearity of the assay, samples were spiked with high concentrations of human IL-12 p70 and diluted with calibrator diluent to produce samples with values within the dynamic range of the assay.

		Cell culture media (n=5)	Serum (n=5)	EDTA plasma (n=5)	Heparin plasma (n=5)	Citrate plasma (n=5)	Urine (n=5)
1:2	Average % of Expected	98	107	107	106	105	105
	Range (%)	95-104	105-111	106-108	105-106	104-106	102-110
1:4	Average % of Expected	100	109	108	105	104	108
	Range (%)	96-109	104-113	106-110	102-108	98-109	104-113
1:8	Average % of Expected	97	108	107	104	103	105
	Range (%)	85-110	102-114	104-111	101-106	101-106	101-110
1:16	Average % of Expected	102	109	109	103	104	108
	Range (%)	89-115	104-119	105-111	95-108	102-105	102-115

SENSITIVITY

The minimum detectable dose (MDD) of human IL-12 p70 is typically less than 5.0 pg/mL.

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

CALIBRATION

This immunoassay is calibrated against a highly purified *Sf* 21-expressed recombinant human IL-12 p70 produced at R&D Systems®.

The NIBSC/WHO 1st International recombinant human IL-12 Standard 95/544 was evaluated in this kit. The dose response curve of the NIBSC Standard 95/544 parallels the Quantikine® standard curve. To convert sample values obtained with the Quantikine® Human IL-12 p70 kit to approximate NIBSC 95/544 International Units, use the equation below.

NIBSC/WHO (95/544) approximate value (IU/mL) = 0.016 x Quantikine® IL-12 p70 value (pg/mL).

SAMPLE VALUES

Serum/Plasma/Urine - Forty samples from apparently healthy volunteers were evaluated for the presence of IL-12 p70 in this assay. No medical histories were available for the donors used in this study. All samples measured less than the lowest human IL-12 p70 standard, 7.8 pg/mL.

Cell Culture Supernates:
NC-37 Burkitt's lymphoma B lymphoblast cells (1 x 10^6 cells/mL) were cultured for 3 days with and without 10 ng/mL PMA and 25 ng/mL calcium ionophore in RPMI 1640 media supplemented with 2 mM L-glutamine, 100 µg/mL penicillin, 100 µg/mL streptomycin, and 1% Nutridoma.

Human peripheral blood mononuclear cells (1 x 10^6 cells/mL) were cultured for 18 hours with and without 0.0075% (wt/vol) SAC (Pansorbin®, EMD Millipore) in RPMI 1640 media supplemented with 1% fetal calf serum.

Condition	NC-37 cells	PBMCs
Stimulated	8.3 pg/mL	33 pg/mL
Unstimulated	ND	ND

ND=Non-detectable

SPECIFICITY

This assay recognizes natural and recombinant human IL-12 p70.

The factors listed below were prepared at 50 ng/mL in calibrator diluent and assayed for cross-reactivity. Preparations of the following factors at 50 ng/mL in a mid-range human IL-12 p70 control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant human:
G-CSF
gp130
IL-6
IL-6 Rα
IL-12/IL-23 p40
IL-12 Rβ1
IL-12 Rβ2
IL-23
IL-27

Recombinant mouse:
IL-6
IL-12/IL-23 p40
IL-12 p70
IL-23
IL-27

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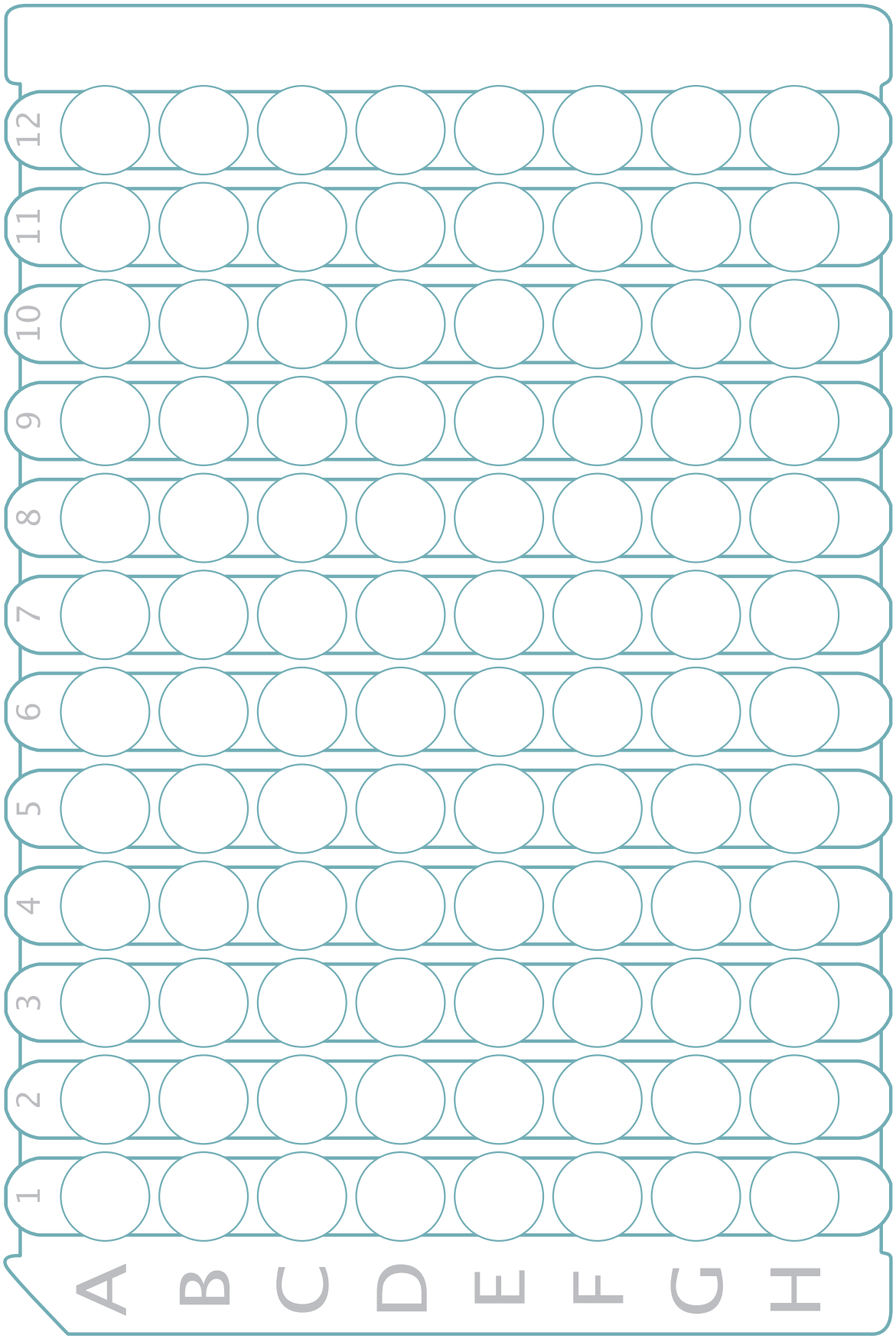
PLATE LAYOUT

Use this plate layout to record standards and samples assayed.



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