Quantikine® ELISA

Porcine IL-12/IL-23 p40 Immunoassay

Catalog Number P1240

For the quantitative determination of porcine Interleukin 12/23 p40 (IL-12/IL-23 p40) concentrations in cell culture supernates, serum, and plasma.

Note: The standard reconstitution method has changed. 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.

TABLE OF CONTENTS

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

INTRODUCTION

Interleukin 12 (IL-12) and Interleukin 23 (IL-23) are secreted heterodimeric glycoproteins that belong to the IL-12 cytokine family (1-4). These two cytokines share a common p40 (40 kDa) subunit, which is disulfide-linked with a p35 (35 kDa) subunit in IL-12, and a p19 (19 kDa) subunit in IL-23. Besides being secreted as a component of IL-12 or IL-23, free p40 monomers and homodimers are also produced (1, 3, 4). Mammalian cells known to express p40 include macrophages, dendritic cells, monocytes, Langerhans cells, neutrophils, keratinocytes, plasmacytoid dendritic cells, and microglia (5-10). From cells that express both the p40 and the IL-12-specific p35 subunit, the amount of free p40 produced is 10-1000-fold higher than the amount of heterodimeric IL-12 produced. IL-12 and IL-23 are important immunoregulatory molecules. They share overlapping but distinct biological activities which promote cell-mediated immunity (3, 4). These activities are mediated by the IL-12 and IL-23 receptor complexes that have a common IL-12 receptor beta 1 subunit (IL-12 Rβ1) partnered with a cytokine-specific IL-12 Rβ2 and IL-23 R subunit, respectively (1, 3, 4). Both monomeric and dimeric free p40 can bind IL-12 Rβ1, but not IL-12 Rβ2 or IL-23 R, and may function as IL-12/IL-23 antagonist. Monomeric p40 binds IL-12 Rβ1 with lower affinity than dimeric p40 and is a less potent antagonist in rodent studies (4, 11-16). Agonistic activities for mouse homodimeric p40 similar to that of heterodimeric IL-12 have also been described, including the induction of nitric oxide expression and NFκB activation in mouse primary microglia and peritoneal macrophages (3). The molecular mechanism for the agonistic effects of homodimeric p40 has not been determined. While porcine p40 self-associates into homodimers, it has not been determined if porcine p40 shows any of the above effects (17).

Mature porcine p40 is a 302 amino acid (aa) heparin-binding glycoprotein that is related to members of the hematopoietin receptor superfamily (1, 4, 17, 18). It contains an N-terminal C2-type Ig-like domain with a C-terminal fibronectin type-III region and a WSxWS-like motif (18). Mature porcine p40 shares 76%, 85%, 84%, 67%, 87%, 86%, and 89% aa sequence identity with guniea pig, canine, human, mouse, feline, equine, and bovine p40, respectively. It shares less than 25% aa sequence identity to porcine p35.

The Quantikine® Porcine IL-12/IL-23 p40 Immunoassay is a 4.5 hour solid-phase ELISA designed to measure porcine IL-12/IL-23 p40 in cell culture supernates, serum, and plasma. It contains CHO cell-expressed recombinant porcine IL-12 and antibodies raised against the recombinant factor. This immunoassay has been shown to accurately quantitate the recombinant factor. Results obtained using natural porcine IL-12/IL-23 p40 showed linear 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 naturally occurring porcine IL-12/IL-23 p40.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for porcine IL-12/IL-23 p40 has been pre-coated onto a microplate. Standards, control, and samples are pipetted into the wells and any IL-12/IL-23 p40 present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for porcine IL-12/IL-23 p40 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 IL-12/IL-23 p40 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.
- If samples generate values higher than the highest standard, further dilute the samples with the appropriate 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.
- For best results, pipette reagents and samples into the center of each well.
- 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
Porcine IL-12/IL-23 p40 Microplate	892484	96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody specific for porcine IL-12/IL-23 p40.	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.*
Porcine IL-12/IL-23 p40 Conjugate	892485	23 mL of a polyclonal antibody specific for porcine IL-12/IL-23 p40 conjugated to horseradish peroxidase with preservatives.	May be stored for up to 1 month at 2-8 °C.*
Porcine IL-12/IL-23 p40 Standard	892486	2 vials of recombinant porcine IL-12 in a buffered protein base with preservatives; lyophilized. *Refer to the vial label for reconstitution volume.*	
Porcine IL-12/IL-23 p40 Control	892487	Recombinant porcine IL-12 in a buffered protein base with preservatives; lyophilized. The assay value of the control should be within the range specified on the label.	
Assay Diluent RD1W	895038	12 mL of a buffered protein base with preservatives.	
Calibrator Diluent RD5Y	895201	21 mL of a buffered protein base with preservatives. *For cell culture supernate samples.*	
Calibrator Diluent RD6-3	895165	21 mL of animal serum with preservatives. *For serum/plasma samples.*	
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.
- 500 mL graduated cylinder.
- Horizontal orbital microplate shaker (0.12" orbit) capable of maintaining a speed of 500 ± 50 rpm.
- Test tubes for dilution of standards and samples.

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

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.

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.*

SAMPLE PREPARATION

Serum and plasma samples require a 4-fold dilution. A suggested 4-fold dilution is 60 µL of sample + 180 µL of Calibrator Diluent RD6-3.

REAGENT PREPARATION

Bring all reagents to room temperature before use.

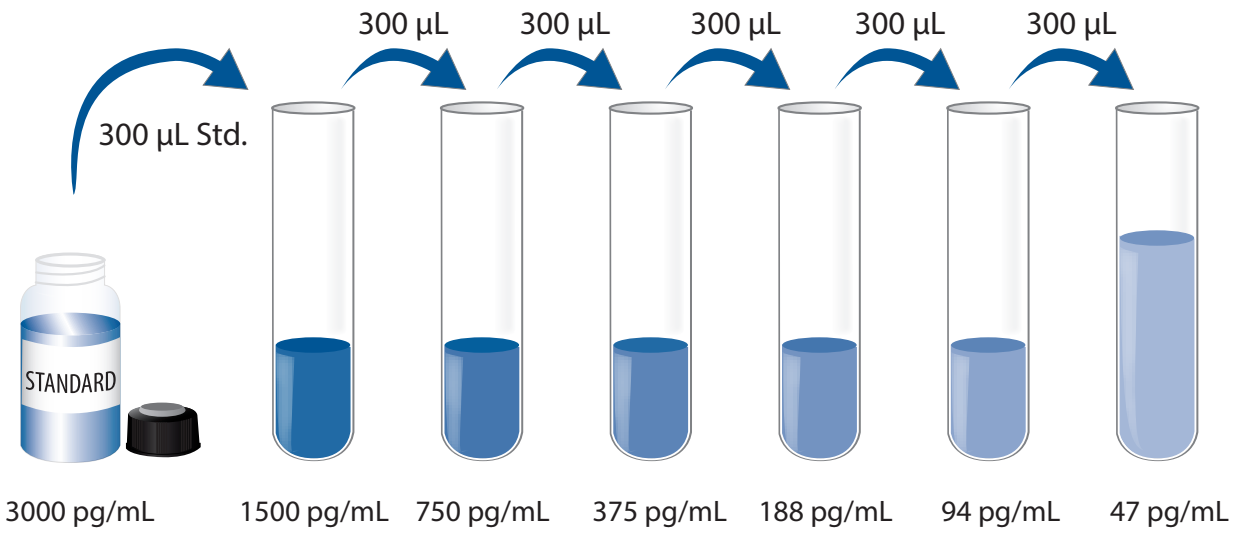
Porcine IL-12/IL-23 p40 Control - Reconstitute the control with 1.0 mL of deionized or distilled water. Mix thoroughly. Assay the control undiluted.

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

Porcine IL-12/IL-23 p40 Standard - **Refer to the vial label for reconstitution volume.** Reconstitute the Porcine IL-12/IL-23 p40 Standard with Calibrator Diluent RD5Y (*for cell culture supernate samples*) or Calibrator Diluent RD6-3 (*for serum/plasma samples*). Do not substitute other diluents. This reconstitution produces a stock solution of 3000 pg/mL. Allow the standard to sit for a minimum of 5 minutes with gentle mixing prior to making dilutions.

Pipette 300 µL of Calibrator Diluent RD5Y (*for cell culture supernate samples*) or Calibrator Diluent RD6-3 (*for serum/plasma samples*) into each tube. Use the stock solution to produce a 2-fold dilution series (below). Mix each tube gently but thoroughly before the next transfer. The undiluted Porcine IL-12/IL-23 p40 Standard (3000 pg/mL) serves as the high standard. The appropriate calibrator diluent 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 RD1W to each well.
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. A plate layout is provided to record standards and samples assayed.
5. Aspirate each well and wash, repeating the process four times for a total of five washes. Wash by filling each well with Wash Buffer (400 µL) using a squirt bottle, manifold dispenser, or auto washer. 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 Porcine IL-12/IL-23 p40 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 120 µL of Substrate Solution to each well. Incubate for 30 minutes at room temperature **on the bench top. Protect from light.**
9. Add 120 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 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.).

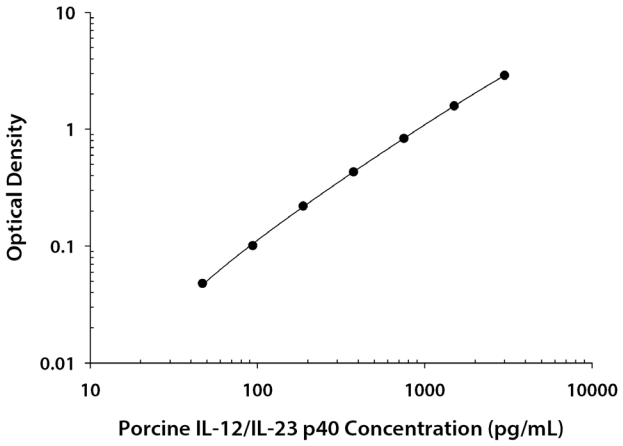
Create a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the log of the porcine IL-12/IL-23 p40 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 prior to assay, the concentration read from the standard curve must be multiplied by the dilution factor.

TYPICAL DATA

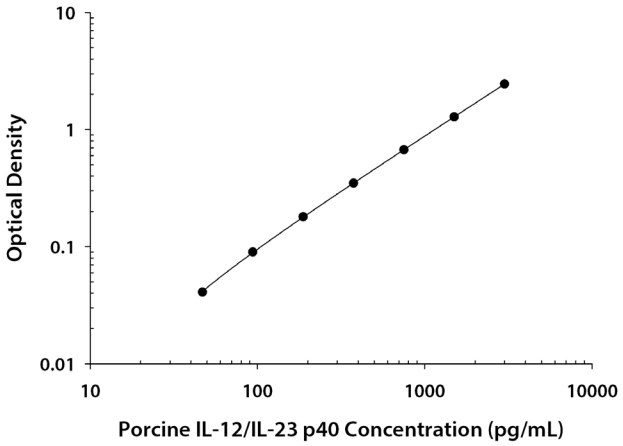
These standard curves are provided for demonstration only. A standard curve should be generated for each set of samples assayed.

CELL CULTURE SUPERNATE ASSAY



(pg/mL)	O.D.	Average	Corrected
0	0.056 0.059	0.058	—
47	0.105 0.106	0.106	0.048
94	0.157 0.161	0.159	0.101
188	0.273 0.282	0.278	0.220
375	0.485 0.493	0.489	0.431
750	0.882 0.904	0.893	0.835
1500	1.613 1.662	1.638	1.580
3000	2.935 2.951	2.943	2.885

SERUM/PLASMA ASSAY



(pg/mL)	O.D.	Average	Corrected
0	0.047 0.050	0.049	—
47	0.089 0.090	0.090	0.041
94	0.138 0.139	0.139	0.090
188	0.227 0.231	0.229	0.180
375	0.393 0.403	0.398	0.349
750	0.714 0.726	0.720	0.671
1500	1.275 1.387	1.331	1.282
3000	2.433 2.562	2.498	2.449

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 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	27	27	27
Mean (pg/mL)	89.8	362	819	88.8	361	795
Standard deviation	7.3	17.7	28.0	9.2	22.9	46.1
CV (%)	8.1	4.9	3.4	10.4	6.3	5.8

SERUM/PLASMA ASSAY

	Intra-Assay Precision			Inter-Assay Precision		
Sample	1	2	3	1	2	3
n	20	20	20	25	25	25
Mean (pg/mL)	115	447	991	110	454	995
Standard deviation	7.4	22.7	33.0	10.1	33.4	64.3
CV (%)	6.4	5.1	3.3	9.2	7.4	6.5

RECOVERY

The recovery of porcine IL-12/IL-23 p40 spiked to levels throughout the range of the assay in various matrices was evaluated.

Sample Type	Average % Recovery	Range
Cell culture supernates (n=4)	104	97-113%
Serum* (n=5)	99	88-109%
EDTA plasma* (n=4)	92	84-99%
Heparin plasma* (n=5)	94	85-105%

*Samples were diluted prior to assay as directed in the Sample Preparation section.

SENSITIVITY

Twenty-nine assays were evaluated and the minimum detectable dose (MDD) of porcine IL-12/IL-23 p40 ranged from 3.5-18.2 pg/mL. The mean MDD was 9.0 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 and/or spiked with high concentrations of porcine IL-12/IL-23 p40 were diluted with the appropriate calibrator diluent to produce samples with values within the dynamic range of the assay.

		Cell culture supernates (n=9)	Serum* (n=5)	EDTA plasma* (n=4)	Heparin plasma* (n=5)
1:2	Average % of Expected	97	106	109	106
	Range (%)	93-101	103-108	106-112	103-110
1:4	Average % of Expected	97	108	111	112
	Range (%)	94-105	105-112	107-115	104-120
1:8	Average % of Expected	100	108	107	108
	Range (%)	89-116	101-112	103-112	102-115
1:16	Average % of Expected	95	105	101	101
	Range (%)	88-109	90-115	95-105	95-111

*Samples were diluted prior to assay as directed in the Sample Preparation section.

CALIBRATION

This immunoassay is calibrated against a highly purified CHO cell-expressed recombinant porcine IL-12 produced at R&D Systems®.

SAMPLE VALUES

Serum/Plasma - Samples were evaluated for detectable levels of porcine IL-12/IL-23 p40 in this assay.

Sample Type	Mean (pg/mL)	% Detectable	Range (pg/mL)
Serum (n=19)	464	84	ND-816
EDTA plasma (n=4)	350	81	ND-522
Heparin plasma (n=5)	383	100	240-548

ND=Non-detectable

Cell Culture Supernates - Porcine peripheral blood cells (5 x 10^6 cells/mL) were cultured for 4 days in DMEM supplemented with 10% fetal bovine serum. Cells were stimulated with 50 ng/mL PMA and 500 ng/mL calcium ionomycin. An aliquot of the cell culture supernate was removed, assayed for porcine IL-12/IL-23 p40, and measured 4808 pg/mL.

SPECIFICITY

This assay recognizes natural and recombinant porcine IL-12/IL-23 p40.

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 porcine IL-12/IL-23 p40 control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant porcine:

GM-CSF
IFN-γ
IL-1α
IL-2
IL-4
IL-6
IL-8

Recombinant mouse:

IL-12
IL-12/IL-23 p40
IL-23
IL-23 R

Recombinant human:

IL-12 p35
IL-12 Rβ1
IL-12 Rβ2
IL-23 p19
IL-23 R

Other recombinants:

rat IL-12
canine IL-12

Natural proteins:

porcine PDGF

Recombinant human IL-12 cross-reacts approximately 3.4% in this assay.

Recombinant human IL-12/IL-23 p40 cross-reacts approximately 4.5% in this assay.

Recombinant feline IL-12 cross-reacts approximately 7.9% in this assay.

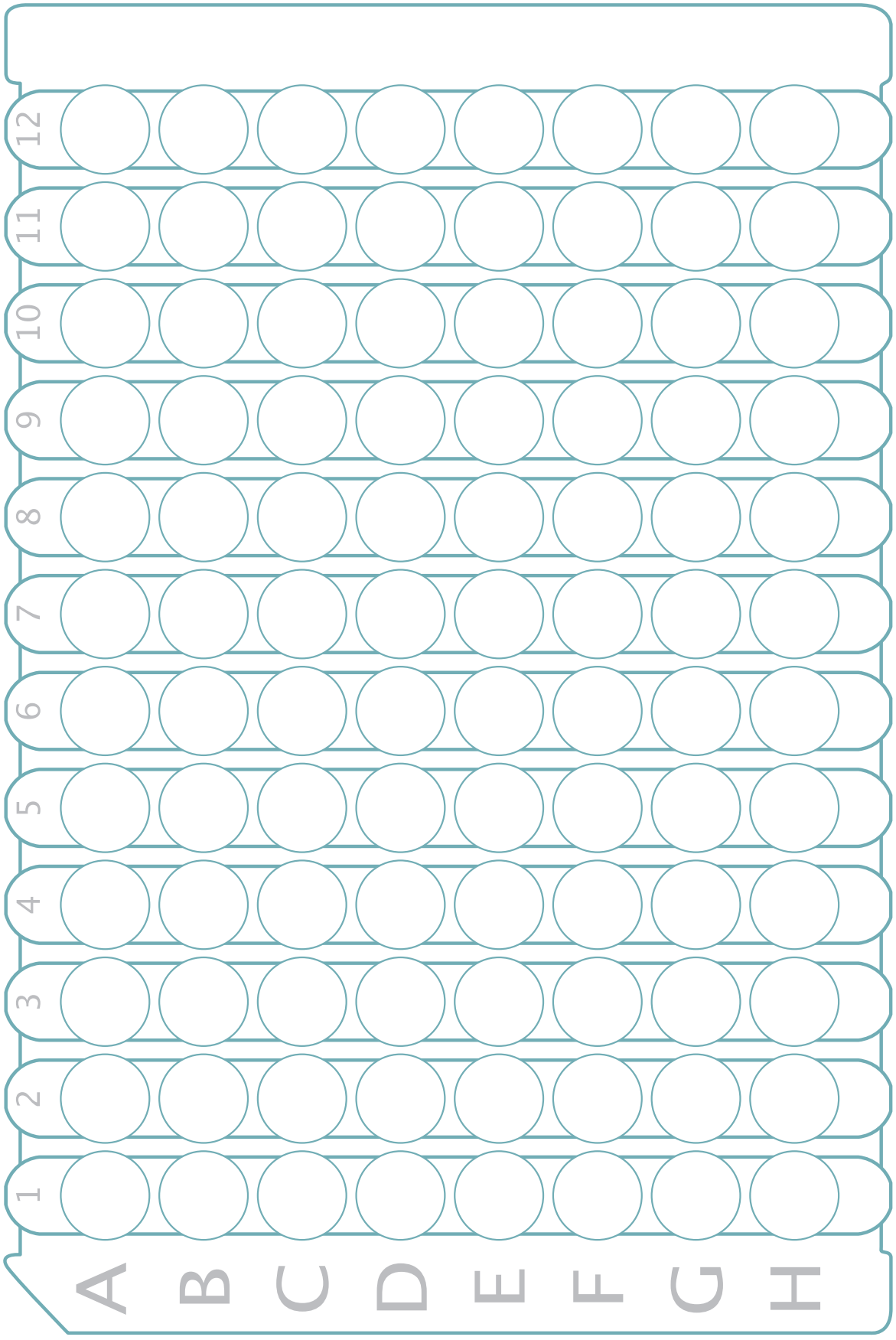
Recombinant feline IL-12/IL-23 p40 cross-reacts approximately 6.7% in this assay.

REFERENCES

1. Langrish, C.L. *et al.* (2004) Immunol. Rev. **202**:96.
2. Novelli, F. and J-L. Casanova (2004) Cytokine Growth Factor Rev. **15**:367.
3. Brombacher, F. *et al.* (2003) Trends Immunol. **24**:207.
4. Trinchieri, G. (2003) Nat. Rev. Immunol. **3**:133.
5. Kato, T. *et al.* (1997) Cell. Immunol. **181**:59.
6. Blotta, M.H. *et al.* (1997) J. Immunol. **158**:5589.
7. Kang, K. *et al.* (1996) J. Immunol. **156**:1402.
8. Romani, L. *et al.* (1997) J. Immunol. **158**:5349.
9. Yawalkar, N. *et al.* (1996) J. Invest. Dermatol. **106**:80.
10. Krug, A. *et al.* (2001) Eur. J. Immunol. **31**:3026.
11. Abdi, K. and S.H. Herrmann (1997) J. Immunol. **159**:3148.
12. Wang, X. *et al.* (1999) Eur. J. Immunol. **29**:2007.
13. Gillessen, S. *et al.* (1995) Eur. J. Immunol. **25**:200.
14. Ling, P. *et al.* (1995) J. Immunol. **154**:116.
15. Chen, L. *et al.* (1997) J. Immunol. **159**:351.
16. Picotti, J.R. *et al.* (1997) J. Immunol. **158**:643.
17. Foss, D.L. and M.P. Murtaugh (1997) Vet. Immunol. Immunopathol. **57**(1-2):121.
18. Hasan, M. *et al.* (1999) J. Immunol. **162**:1064.

PLATE LAYOUT

Use this plate layout to record standards and samples assayed.



NOTES

NOTES