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R&D SYSTEMS

Quantikine™ ELISA

Mouse/Rat IL-22 Immunoassay

Catalog Number M2200

For the quantitative determination of mouse/rat Interleukin 22 (IL-22) 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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Manufactured and Distributed by:

USA R&D Systems, Inc.
614 McKinley Place NE, Minneapolis, MN 55413
TEL: 800 343 7475 612 379 2956
FAX: 612 656 4400
E-MAIL: info@bio-techne.com

Distributed by:

Europe | Middle East | Africa Bio-Techne Ltd.
19 Barton Lane, Abingdon Science Park
Abingdon OX14 3NB, UK
TEL: +44 (0)1235 529449
FAX: +44 (0)1235 533420
E-MAIL: info.emea@bio-techne.com

China Bio-Techne China Co., Ltd.
Unit 1901, Tower 3, Raffles City Changning Office,
1193 Changning Road, Shanghai PRC 200051
TEL: +86 (21) 52380373 (400) 821-3475
FAX: +86 (21) 52371001
E-MAIL: info.cn@bio-techne.com

INTRODUCTION

IL-22, also known as IL-TIF (IL-10 related T cell-derived Inducible Factor), is a 25-40 kDa glycoprotein that belongs to the IFN/IL-10 cytokine family (1-3). It is a six α-helix bundle cytokine that contains two intrachain disulfide bonds. Mouse IL-22 is synthesized as a 179 amino acid (aa) precursor with a 33 aa signal sequence and a 146 aa mature segment (1, 4). In some mouse strains, a second copy of the IL-22 gene, designated IL-22β, has been identified. Mouse IL-22β gene, which likely arises from gene duplication, encodes a protein that differs from the mature mouse IL-22(α) in three aa residues. It has not been determined if mouse IL-22β is actually expressed (4, 7). At physiological concentration, IL-22 exists as a monomer in solution. At high concentrations, IL-22 can also form nondisulfide-linked homodimers (5). Mature mouse IL-22 shares 92% and 81% amino acid sequence identity with rat and human IL-22, respectively. IL-22 is primarily expressed by activated T cells and NK cells (1, 2, 6, 7). Among $CD4^+$ T cells, IL-22 is most highly expressed in Th1 cells (8).

IL-22 signals via the heteromeric IL-22 receptor complex that is composed of IL-22 R1 and IL-10 Rβ, both of which belong to the class II cytokine receptor (CRF2) family (9-14). These proteins are type I transmembrane glycoproteins that contain two tandem fibronectin type III (FNIII) repeats in their extracellular domains. Monomeric IL-22 first binds with high-affinity to IL-22 R, and subsequently recruits IL-10 Rβ to form a complex that transduces IL-22 signal through pathways involving Janus kinases and STATs (7). IL-10 Rβ is ubiquitously expressed and functions also as a subunit in receptor complexes for IL-10, IL-26, IL-28 and IL-29 (7). IL-22 R1 expression is limited to the epithelium and parenchymal cells in various organs including the skin, kidney, and tissues of the digestive and respiratory system (7, 15-17). IL-22 R1 also functions as a subunit in the receptor complexes for IL-20 and IL-24. In addition to the membrane IL-22 R1, IL-22 binds to a secreted IL-22 binding protein (IL-22 BP), also known as IL-22 Rα2 (7, 18, 19). IL-22 BP is the product of a distinct gene and is predicted to also have two FNIII repeats. Unlike IL-22 R1, IL-22 BP does not bind other members of the IL-10 family (2, 7). *In vitro*, IL-22 BP has been shown to be an IL-22 antagonist. *In vivo*, IL-22 BP has been suggested to act as a carrier of IL-22 for transport to target cells (7).

IL-22 is important in innate immunity and exerts its effects on tissue cells where IL-22 R1 is expressed (7). In skin keratinocytes, IL-22 regulates the expression of genes responsible for antimicrobial defense, as well as genes required for increased cell proliferation and mobility, which are necessary for wound healing and tissue reorganization (3, 7, 16). In the liver and intestine, IL-22 induces the production of acute phase response proteins (2, 6, 7, 15). It also protects against liver injury (7, 20). In the pancreas, IL-22 has been shown to upregulate the expression of pancreatitis-associated protein 1 (PAP1) and osteopontin, which play important roles in the protection against tissue injury (2, 7, 21).

The Quantikine™ Mouse/Rat IL-22 Immunoassay is a 4.5 hour solid-phase ELISA designed to measure mouse and rat IL-22 in cell culture supernates, serum, and plasma. It contains *E. coli*-expressed recombinant mouse IL-22 and antibodies raised against the recombinant factor. This immunoassay has been shown to accurately quantitate the recombinant factor. Results obtained using natural mouse or rat IL-22 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 mouse and rat IL-22.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A polyclonal antibody specific for mouse/rat IL-22 has been pre-coated onto a microplate. Standards, control, and samples are pipetted into the wells and any mouse or rat IL-22 present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for mouse/rat IL-22 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 mouse or rat IL-22 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 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 IL-22 Microplate	893208	96 well polystyrene microplate (12 strips of 8 wells) coated with a polyclonal antibody specific for mouse/rat IL-22.	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 IL-22 Conjugate	893209	21 mL of a polyclonal antibody specific for mouse/rat IL-22 conjugated to horseradish peroxidase with preservatives.	May be stored for up to 1 month at 2-8 °C.*
Mouse/Rat IL-22 Standard	893210	2 vials of recombinant mouse IL-22 in a buffered protein base with preservatives; lyophilized. *Refer to the vial label for reconstitution volume.*	
Mouse/Rat IL-22 Control	893211	Recombinant mouse IL-22 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 RD1-17	895433	11 mL of a buffered protein base with preservatives.	
Calibrator Diluent RD5-27	895395	21 mL of a buffered protein base with preservatives. *For cell culture supernate samples.*	
Calibrator Diluent RD5V	895425	21 mL of a buffered protein base 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 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.

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.

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.

Note: *Citrate plasma samples have not been validated for use in this assay.*
Grossly hemolyzed or lipemic samples may not be suitable for use in this assay.

SAMPLE PREPARATION

Serum and plasma samples require a 2-fold dilution prior to assay. A suggested 2-fold dilution is 70 µL of sample + 70 µL of Calibrator Diluent RD5V.

REAGENT PREPARATION

Bring all reagents to room temperature before use.

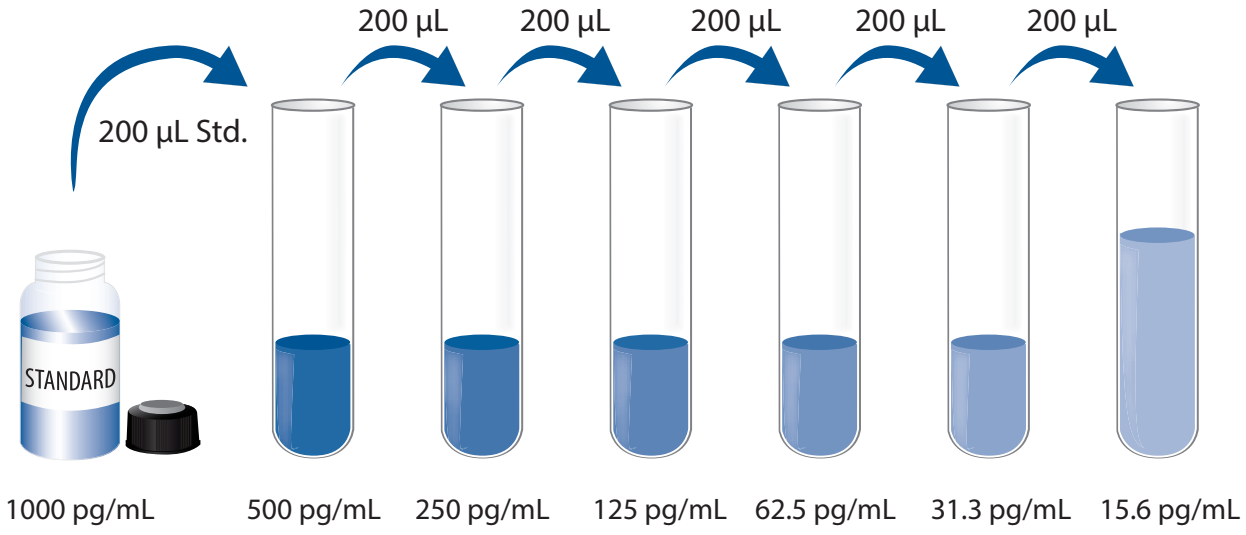
Mouse/Rat IL-22 Control - Reconstitute the control with 1.0 mL of deionized or distilled water. Mix thoroughly. Assay the control undiluted.

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

Mouse/Rat IL-22 Standard - **Refer to the vial label for reconstitution volume.** Reconstitute the Mouse/Rat IL-22 Standard with Calibrator Diluent RD5-27 (*for cell culture supernate samples*) or Calibrator Diluent RD5V (*for serum/plasma samples*). Do not substitute other diluents. This reconstitution produces a stock solution of 1000 pg/mL. Allow the stock solution to sit for a minimum of 5 minutes with gentle mixing prior to making dilutions.

Pipette 200 µL of the appropriate calibrator diluent into each tube. Use the stock solution to produce a dilution series (below). Mix each tube gently but thoroughly before the next transfer. The undiluted Mouse/Rat IL-22 Standard (1000 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 100 µL of Assay Diluent RD1-17 to each well.
4. Add 50 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 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 Mouse/Rat IL-22 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 benchtop. 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.

CALCULATION OF RESULTS

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

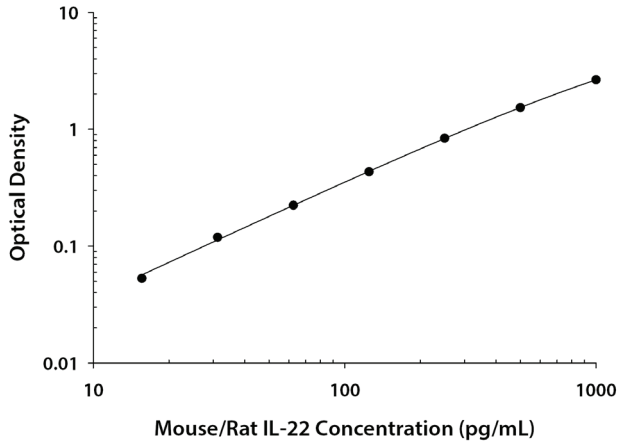
Create a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the log of the mouse/rat IL-22 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

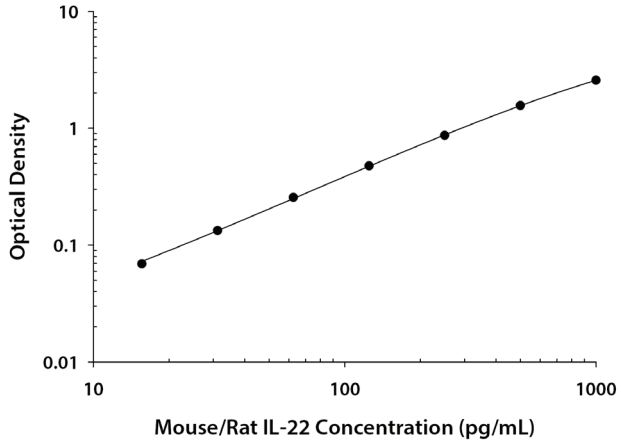
These standard curves are provided for demonstration only. A standard curve should be generated for each set of samples assayed.

CELL CULTURE SUPERNATE ASSAY



(pg/mL)	O.D.	Average	Corrected
0	0.036 0.042	0.039	—
15.6	0.089 0.095	0.092	0.053
31.3	0.154 0.161	0.158	0.119
62.5	0.259 0.264	0.262	0.223
125	0.465 0.477	0.471	0.432
250	0.873 0.878	0.876	0.837
500	1.555 1.589	1.572	1.533
1000	2.601 2.766	2.684	2.645

SERUM/PLASMA ASSAY



(pg/mL)	O.D.	Average	Corrected
0	0.037 0.038	0.038	—
15.6	0.104 0.109	0.107	0.069
31.3	0.167 0.174	0.171	0.133
62.5	0.292 0.296	0.294	0.256
125	0.509 0.519	0.514	0.476
250	0.904 0.906	0.905	0.867
500	1.599 1.602	1.601	1.563
1000	2.605 2.610	2.608	2.570

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	35	35	35
Mean (pg/mL)	45.9	129	628	43.9	122	596
Standard deviation	1.9	7.5	22.1	3.4	6.7	25.2
CV (%)	4.2	5.8	3.5	7.7	5.5	4.2

SERUM/PLASMA ASSAY

	Intra-Assay Precision			Inter-Assay Precision		
Sample	**1**	**2**	**3**	**1**	**2**	**3**
n	20	20	20	34	34	34
Mean (pg/mL)	44.4	117	692	45.4	128	662
Standard deviation	2.0	7.0	25.7	4.8	7.5	35.5
CV (%)	4.4	6.0	3.7	10.5	5.9	5.4

RECOVERY

The recovery of IL-22 spiked to three levels throughout the range of the assay in various matrices was evaluated.

Mouse Samples	Average % Recovery	Range
Cell culture supernates (n=4)	98	93-103%
Serum* (n=4)	91	84-99%
EDTA plasma* (n=4)	95	86-106%
Heparin plasma* (n=4)	94	83-102%

Rat Samples	Average % Recovery	Range
Cell culture supernates (n=4)	98	91-102%
Serum* (n=4)	96	90-107%
EDTA plasma* (n=4)	97	91-111%
Heparin plasma* (n=4)	93	88-101%

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

LINEARITY

To assess the linearity of the assay, samples spiked with high concentrations of IL-22 in each matrix were diluted with the appropriate calibrator diluent and assayed.

Mouse Samples		Cell culture supernates (n=4)	Serum* (n=4)	EDTA plasma* (n=4)	Heparin plasma* (n=4)
1:2	Average % of Expected	100	103	103	100
	Range (%)	97-102	97-107	102-104	90-105
1:4	Average % of Expected	101	105	103	102
	Range (%)	98-104	98-115	101-106	99-106
1:8	Average % of Expected	97	104	106	99
	Range (%)	95-99	100-111	105-106	93-109
1:16	Average % of Expected	88	100	105	93
	Range (%)	83-90	94-106	103-109	86-111

Rat Samples		Cell culture supernates (n=4)	Serum* (n=4)	EDTA plasma* (n=4)	Heparin plasma* (n=4)
1:2	Average % of Expected	100	105	106	107
	Range (%)	94-104	99-112	98-111	106-109
1:4	Average % of Expected	103	109	101	107
	Range (%)	98-108	103-119	97-104	105-108
1:8	Average % of Expected	99	105	97	100
	Range (%)	99-99	101-114	92-101	96-103
1:16	Average % of Expected	89	98	86	92
	Range (%)	88-92	93-106	81-92	87-94

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

SENSITIVITY

Forty-seven assays were evaluated and the minimum detectable dose (MDD) of mouse/rat IL-22 ranged from 0.76-8.2 pg/mL. The mean MDD was 3.2 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 *E. coli*-expressed recombinant mouse IL-22 produced at R&D Systems®.

SAMPLE VALUES

Serum/Plasma - Samples were evaluated for the presence of mouse/rat IL-22 in this assay. Samples were diluted prior to assay as directed in the Sample Preparation section.

Mouse Samples	Mean of Detectable (pg/mL)	% Detectable	Range (pg/mL)
Serum (n=20)	101	85	ND-301
EDTA plasma (n=20)	139	60	ND-662
Heparin plasma (n=20)	66	90	ND-188

Rat Samples	Mean of Detectable (pg/mL)	% Detectable	Range (pg/mL)
Serum (n=15)	137	80	ND-296
EDTA plasma (n=20)	187	90	ND-1145
Heparin plasma (n=20)	101	75	ND-204

ND=Non-detectable

Cell Culture Supernates:

Two mouse spleens were cultured for 4 days in RPMI 1640 supplemented with 10% fetal bovine serum, 50 µM β-mercaptoethanol, 2 mM L-glutamine, and 100 µg/mL of streptomycin sulfate. The cells were stimulated with 100 ng/mL of recombinant mouse IFN-γ and 1 µg/mL of LPS. An aliquot of the cell culture supernate was removed, tested for IL-22, and measured 532 pg/mL.

One rat spleen was cultured for 18 hours in RPMI 1640 supplemented with 10% fetal bovine serum, 50 µM β-mercaptoethanol, 2 mM L-glutamine, and 100 µg/mL of streptomycin sulfate. The cells were stimulated with 10 µg/mL of PHA and 10 ng/mL of PMA. An aliquot of the cell culture supernate was removed, tested for IL-22, and measured 1105 pg/mL.

SPECIFICITY

This assay recognizes natural and recombinant mouse and rat IL-22.

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 mouse/rat IL-22 control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant mouse:
IL-1ra
IL-1 R2
IL-5 Rα
IL-6
IL-10
IL-10 Rα
IL-11
IL-11 Rα
IL-12
IL-12 p35
IL-12 p40
IL-12 p40 (dimer)
IL-13 Rα
IL-17 R
IL-18
IL-20 Rα
IL-22BP
IL-23
IL-28

Recombinant rat:
CNTF
IL-1α
IL-1β
IL-1 Rα
IL-1 R6
IL-2
IL-3
IL-4
IL-5
IL-6
IL-10

Recombinant human:
IL-6
IL-10
IL-10 Rα
IL-10 Rβ
IL-12
IL-12 p40
IL-19
IL-20
IL-20 Rα
IL-20 Rβ
IL-22 R
IL-24
IL-28A

Other recombinants:
canine IL-10
porcine IL-10

Recombinant rat IL-22 cross-reacts approximately 60% in this assay.

Recombinant human IL-22 cross-reacts approximately 30% in this assay.

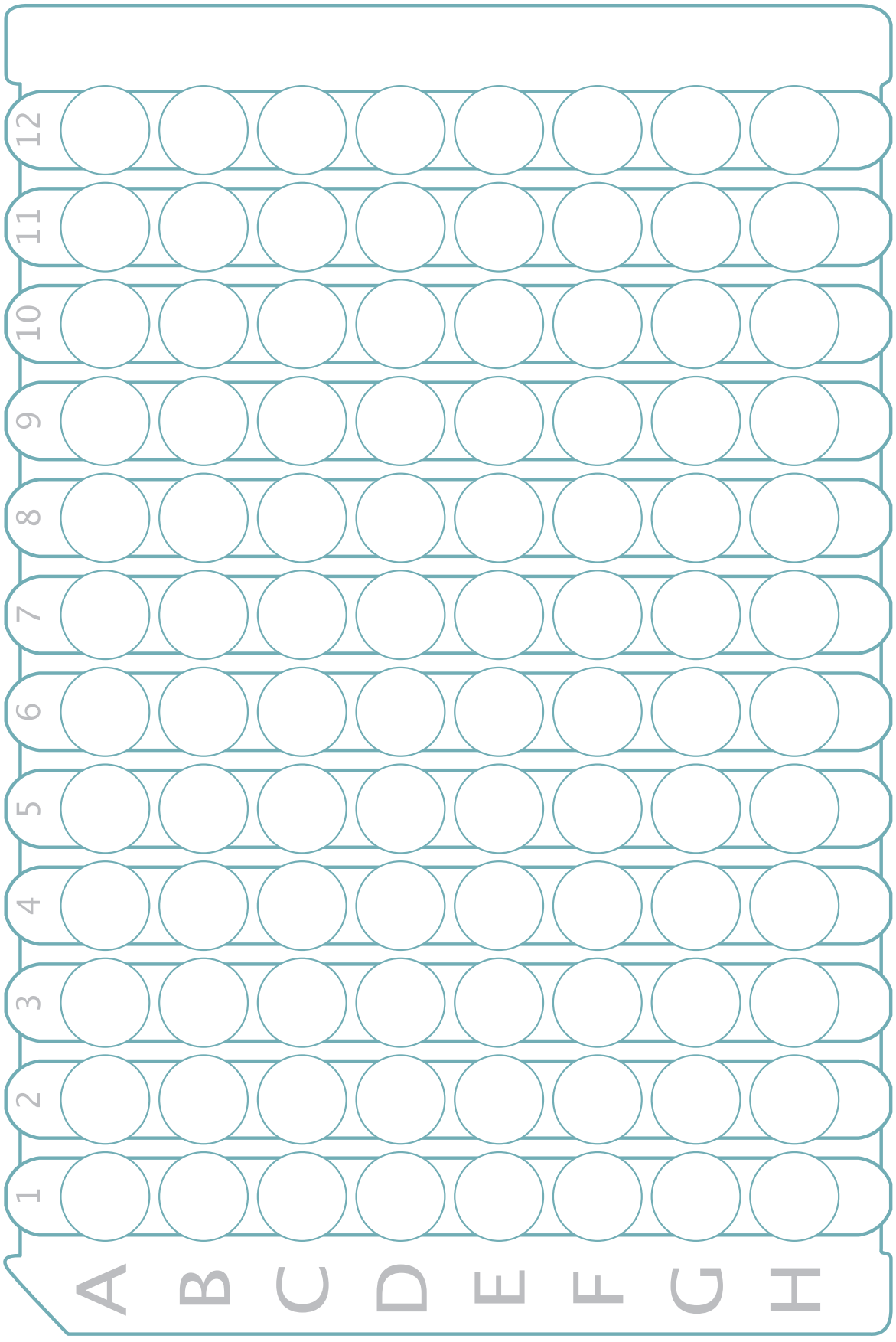
Recombinant human IL-22BP interferes at concentrations > 25 ng/mL.

REFERENCES

1. Dumoutier, L. *et al.* (2000) J. Immunol. **164**:1814.
2. Gurney, A.L. (2004) Int. Immunopharmacol. **4**:669.
3. Wolk, K. *et al.* (2006) Eur. J. Immunol. **36**:1309.
4. Dumoutier, L. *et al.* (2000) Genes Immun. **1**:488.
5. Nagem, R.A.P. *et al.* (2002) Structure **10**:1051.
6. Nagalakshmi, M.L. *et al.* (2004) Int. Immunopharmacol. **4**:679.
7. Wolk, K. and R. Sabat (2006) Cytokine and Growth Factor Reviews **17**:367.
8. Liang, S.C. *et al.* (2006) J. Exp. Med. **203**:2271.
9. Xie, M-H. *et al.* (2000) J. Biol. Chem. **275**:31335.
10. Li, J. *et al.* (2004) Int. Immunopharmacol. **4**:693.
11. Kotenko, S.V. and J.A. Langer (2004) Int. Immunopharmacol. **4**:593.
12. Kotenko, S.V. *et al.* (2001) J. Biol. Chem. **276**:2725.
13. Tachiiri, A. *et al.* (2003) Genes Immun. **4**:153.
14. Spencer, S.D. *et al.* (1998) J. Exp. Med. **187**:571.
15. Dumoutier, L. *et al.* (2000) Proc. Natl. Acad. Sci. USA **97**:10144.
16. Wolk, K. *et al.* (2004) Immunity **21**:241.
17. Nagalakshmi, M.L. *et al.* (2004) Int. Immunopharmacol. **4**:577.
18. Wei, C-C. *et al.* (2003) Genes Immun. **4**:204.
19. Weiss, B. *et al.* (2004) Genes Immun. **5**:330.
20. Radaeva, S. *et al.* (2004) Hepatology **39**:1332.
21. Aggarwal, S. *et al.* (2001) J. Interf. Cytokine Res. **21**:1047.

PLATE LAYOUT

Use this plate layout to record standards and samples assayed.



NOTES