Quantikine® HS ELISA

Human IL-10 Immunoassay

Catalog Number HS100C
SS100C
PHS100C

For the quantitative determination of human Interleukin 10 (IL-10) concentrations in 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.

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 10 (IL-10), also known as cytokine synthesis inhibitory factor (CSIF), is the charter member of the IL-10 family of α-helical cytokines that also includes IL-19, IL-20, IL-22, IL-24, and IL-26/AK155 (1, 2). Mature human IL-10 is an 18 kDa molecule with one potential N-linked glycosylation site and four cysteines which form two intrachain disulfide bridges (3, 4). It is expressed as a noncovalently associated homodimer (5). Mature human IL-10 shares 72-86% amino acid sequence identity with bovine, canine, equine, feline, mouse, ovine, porcine, and rat IL-10. IL-10 is secreted by many activated hematopoietic cell types, hepatic stellate cells, keratinocytes, and placental cytotrophoblasts (1, 6-8).

IL-10 mediates its biological activities through a heteromeric receptor complex that is composed of the type II cytokine receptor subunits IL-10 Rα/IL-10R1 and IL-10 Rβ/IL-10R2. These molecules each contain two fibronectin type-III domains in their extracellular region but do not contain WXSWS motifs that are characteristic of type I cytokine receptors (2). The IL-10 dimer binds to two IL-10 Rα chains, resulting in recruitment of two IL-10 Rβ chains and activation of a signaling cascade involving JAK1, TYK2, and STAT3 (9). IL-10 Rα is a 110 kDa transmembrane glycoprotein that is expressed on lymphocytes, NK cells, macrophages, monocytes, intestinal epithelial cells, cytotrophoblasts, astrocytes, and activated hepatic stellate cells (6, 8, 10-13). IL-10 Rβ is a widely expressed 75 kDa transmembrane glycoprotein that does not bind IL-10 by itself but is required for signal transduction (9, 14). IL-10 Rβ also associates with IL-20 Rα, IL-22 Rα, or IL-28 Rα to form the receptor complexes for IL-22, IL-26, IL-28, and IL-29 (15-17).

The involvement of IL-10 in immunoregulation includes both positive and negative effects. It promotes phagocytic uptake and Th2 responses but suppresses antigen presentation and Th1 proinflammatory responses (1). IL-10 is a critical molecule in the control of viral infections as well as allergic and autoimmune inflammation (18-20). Serum, peritoneal fluid, and saliva levels of IL-10 are elevated in melanoma, ovarian cancer, and Sjogren's syndrome (21-23). IL-10 levels are decreased in the serum during preeclampsia and in the seminal fluid of infertile men (24, 25). Polymorphisms of IL-10 are associated with the development of autoimmunity, viral infection, and cancer, while polymorphisms of IL-10 Rα are associated with schizophrenia (26-29).

The Quantikine HS Human IL-10 Immunoassay is a 5.5 hour solid phase ELISA designed to measure IL-10 in serum and plasma. It contains *Sf* 21-expressed recombinant human IL-10 and antibodies raised against the recombinant factor. This immunoassay has been shown to accurately quantitate recombinant human IL-10. Results obtained using natural human IL-10 showed linear curves that are 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-10.

PRINCIPLE OF THE ASSAY

DUE TO THE HIGH SENSITIVITY OF THIS KIT, PLEASE NOTE THE FOLLOWING PRECAUTIONS.

- **Alkaline phosphatase is detectable in saliva.** Take precautionary measures (e.g. wear a mask and gloves) to protect reagents during preparation and use and while running the assay (reagent preparation through the addition of Stop Solution).
- Inorganic phosphate is a strong competitive inhibitor of alkaline phosphatase; avoid the use of PBS based wash buffers and other sources of inorganic phosphate contamination.
- Use separate pipettes and glassware for dispensing all reagents to avoid cross-contamination.
- Careful washing of the microplate is essential to minimize nonspecific binding of the conjugate.

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for human IL-10 has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any IL-10 present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked monoclonal antibody specific for human IL-10 is added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, a substrate solution is added to the wells. After an incubation period, an amplifier solution is added to the wells and color develops in proportion to the amount of IL-10 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.
- Although this kit has been designed to eliminate matrix problems, some samples may exist that give falsely elevated values when assayed neat. If either serum or plasma samples generate values that are suspiciously high or higher than the highest standard, dilute the samples in 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

- To ensure accurate results, bring liquids to room temperature and mix to homogeneity prior to pipetting or aliquoting.
- When mixing 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.
- 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.
- To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
- Neither the addition of the Substrate Solution nor the Stop Solution will result in a color change.
- Addition of the Amplifier Solution will result in a color change (colorless to shades of maroon).
- Amplifier Solution and Stop Solution should be added to the plate in the same order as 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 # HS100C	CATALOG # SS100C	DESCRIPTION	STORAGE OF OPENED/ RECONSTITUTED MATERIAL
Human IL-10 HS Microplate	893857	1 plate	6 plates	96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody specific for human IL-10.	Return unused wells to the foil pouch containing the desiccant pack. Reseal along entire edge of zip-seal. May be stored for up to 1 month at 2-8 °C.*
Human IL-10 HS Standard	893859	1 vial	6 vials	Recombinant human IL-10 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 ≤ -70 °C. Avoid repeated freeze-thaw cycles.*
Human IL-10 HS Conjugate	893858	1 vial	6 vials	21 mL/vial of a monoclonal antibody specific for human IL-10 conjugated to alkaline phosphatase with preservatives.	May be stored for up to 1 month at 2-8 °C.*
Assay Diluent RD1-10	895168	1 vial	6 vials	6 mL/vial of a buffered protein base with preservatives. *May contain a precipitate. Mix well before and during use. Take precautions to avoid microbial contamination.*	
Calibrator Diluent RD6-10	895468	1 vial	6 vials	21 mL/vial of buffered protein base with preservatives.	
Wash Buffer Concentrate	895188	1 vial	6 vials	100 mL/vial of a 10-fold concentrated solution of buffered surfactant with preservatives. *May turn yellow over time.*	
Stop Solution	895032	1 vial	6 vials	6 mL/vial of 2 N sulfuric acid.	
Substrate	895884	1 vial	6 vials	Lyophilized NADPH with stabilizers.	**Store reconstituted substrate and amplifier solutions in an upright position** for up to 1 month at ≤ -20 °C in a manual defrost freezer.* Avoid repeated freeze-thaw cycles.
Substrate Diluent	895885	1 vial	6 vials	7 mL/vial of buffered solution with stabilizers.	
Amplifier	895886	1 vial	6 vials	Lyophilized amplifier enzymes with stabilizers.	
Amplifier Diluent	895887	1 vial	6 vials	7 mL/vial of buffered solution containing INT-violet with stabilizer.	
Plate Sealers	N/A	8 strips	48 strips	Adhesive strips.	

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

HS100C contains sufficient materials to run an ELISA on one 96 well plate.
SS100C (SixPak) contains sufficient materials to run ELISAs on six 96 well plates.

This kit is also available in a PharmPak (R&D Systems, Catalog # PHS100C). 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 490 nm, with dual wavelength correction set at 650 nm or 690 nm.
- Pipettes and pipette tips.
- Deionized or distilled water.
- Squirt bottle, manifold dispenser, or automated microplate washer.
- 1000 mL graduated cylinder.
- Horizontal orbital microplate shaker (0.12" orbit) capable of maintaining a speed of 500 ± 50 rpm.
- **Polypropylene** test tubes for dilution of standards.
- Human IL-10 Controls (optional; R&D Systems, Catalog # QC41).

PRECAUTIONS

Some components of this kit contain sodium azide which may react with lead and copper plumbing to form explosive metallic azides. Flush with large volumes of water during disposal.

Alkaline phosphatase is detectable in saliva. Take precautionary measures (e.g. wear a mask and gloves) to protect reagents during preparation and use and while running the assay (reagent preparation through the addition of Stop Solution).

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.

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.

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 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.

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

REAGENT PREPARATION

Bring all reagents to room temperature before use.

Note: Alkaline phosphatase is detectable in saliva. *Take precautionary measures to protect reagents (e.g. wear a mask and gloves).*

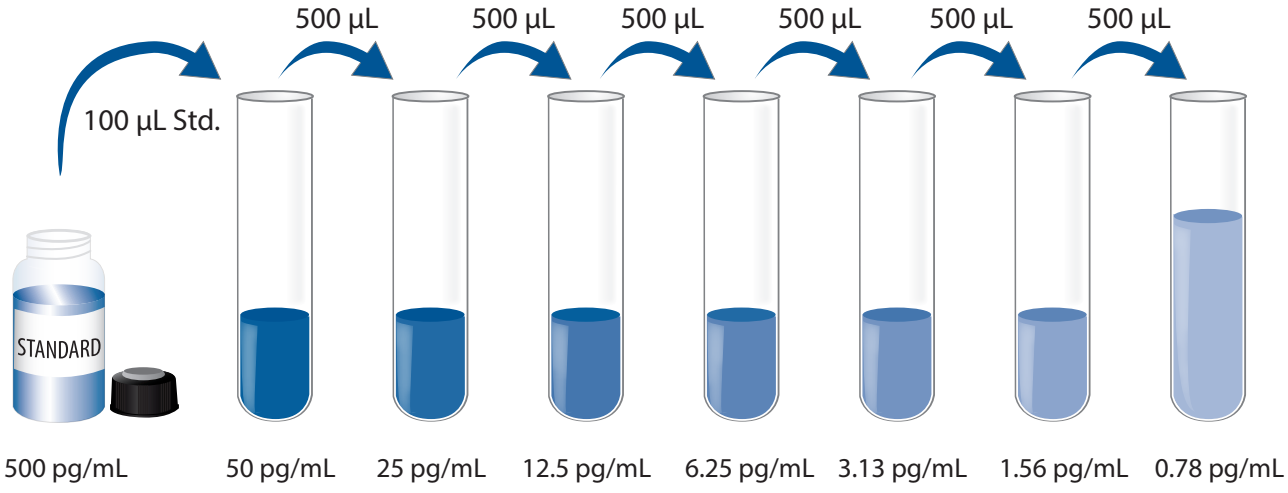
Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Add 100 mL of Wash Buffer Concentrate to deionized or distilled water to prepare 1000 mL of Wash Buffer.

Substrate Solution - Reconstitute the lyophilized Substrate in 6 mL of Substrate Diluent at least 10 minutes before use. **Re-stopper and re-cap the vial**, and mix thoroughly. Avoid contamination.

Amplifier Solution - Reconstitute the lyophilized Amplifier in 6 mL of Amplifier Diluent at least 10 minutes before use. **Re-stopper and re-cap the vial**, and mix thoroughly. Avoid contamination.

Human IL-10 HS Standard - **Refer to the vial label for reconstitution volume.** Reconstitute the Human IL-10 HS Standard with Calibrator Diluent RD6-10. 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.

Use polypropylene tubes. Pipette 900 µL of Calibrator Diluent RD6-10 into the 50 pg/mL tube. Pipette 500 µL into the remaining tubes. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The 50 pg/mL standard serves as the high standard. Calibrator Diluent RD6-10 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.

Note: *Alkaline phosphatase is detectable in saliva. Take precautionary measures to protect reagents (e.g. wear a mask and gloves).*

1. Prepare all reagents and working standards 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 RD1-10 to each well. *Assay Diluent RD1-10 may contain a precipitate. Mix well before and during use. Take precautions to avoid microbial contamination.*
4. Add 200 µL of Standard, sample, or control 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. Wash.

Wash Procedure

   a. Remove liquid from the wells by inverting the plate and decanting the contents.
   b. Remove excess liquid by grasping the plate firmly and smartly rapping the inverted plate on a clean paper towel at least 5 times.
   c. Fill each well with 400 µL of Wash Buffer using an autowasher, squirt bottle, or a manifold dispenser.
   d. Remove liquid from the wells by inverting the plate and decanting the contents.
   e. Repeat steps b-d 5 times for a total of 6 washes. After the last wash, smartly rap the inverted plate on a clean paper towel at least 10 times to remove excess Wash Buffer.

6. Add 200 µL of Human IL-10 HS Conjugate to each well. Cover with a new adhesive strip. Incubate for 2 hours at room temperature on the shaker.
7. Repeat the wash as in step 5.
8. Add 50 µL of Substrate Solution to each well. Cover with a new adhesive strip. Incubate for 1 hour at room temperature on the shaker. **Do not wash the plate.**
9. Add 50 µL of Amplifier Solution to each well. Cover with a new adhesive strip. Incubate for 30 minutes at room temperature on the shaker. **Note:** *Addition of Amplifier Solution initiates color development.*
10. Add 50 µL of Stop Solution to each well. Addition of Stop Solution does not affect the color in the wells.
11. Determine the optical density of each well within 30 minutes, using a microplate reader set at 490 nm. If wavelength correction is available, set to 650 nm or 690 nm. If wavelength correction is not available, subtract readings at 650 nm or 690 nm from the readings at 490 nm. This subtraction will correct for optical imperfections in the plate. Readings made directly at 490 nm without correction may be higher and less accurate.

CALCULATION OF RESULTS

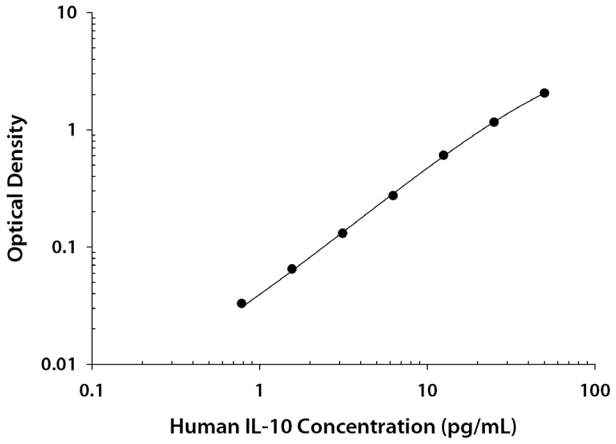
Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density (O.D.).

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-10 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

The following standard curve is provided for demonstration only. A standard curve must be generated for each set of samples assayed.



(pg/mL)	O.D.	Average	Corrected
0	0.016 0.016	0.016	—
0.78	0.048 0.050	0.049	0.033
1.56	0.080 0.082	0.081	0.065
3.13	0.146 0.148	0.147	0.131
6.25	0.283 0.297	0.290	0.274
12.5	0.613 0.632	0.623	0.607
25	1.109 1.242	1.176	1.160
50	2.028 2.113	2.071	2.055

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 thirty-five 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	35	35	35
Mean (pg/mL)	2.36	10.7	21.0	1.68	10.4	20.9
Standard deviation	0.22	0.62	0.97	0.22	0.81	1.78
CV (%)	9.3	5.8	4.6	13.1	7.8	8.5

RECOVERY

The recovery of human IL-10 spiked to three different levels throughout the range of the assay in various matrices was evaluated.

Sample Type	Average % Recovery	Range
Serum (n=4)	100	94-108%
EDTA plasma (n=4)	90	84-99%
Citrate plasma (n=4)	93	81-106%

LINEARITY

To assess the linearity of the assay, samples were spiked with high concentrations of human IL-10 in various matrices and diluted with Calibrator Diluent to produce samples with values within the dynamic range of the assay.

		Serum (n=4)	EDTA plasma (n=4)	Citrate plasma (n=4)
1:2	Average % of Expected	105	103	106
	Range (%)	100-112	100-106	102-111
1:4	Average % of Expected	101	108	106
	Range (%)	95-107	104-118	100-112
1:8	Average % of Expected	98	99	97
	Range (%)	90-107	93-110	84-106

SENSITIVITY

Twenty-three assays were evaluated and the minimum detectable dose (MDD) of human IL-10 ranged from 0.03-0.17 pg/mL. The mean MDD was 0.09 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 highly purified *Sf* 21-expressed recombinant human IL-10 produced at R&D Systems.

The NIBSC/WHO recombinant human IL-10 Reference Reagent 93/722 was evaluated in this kit. The dose response curve of the reference standard 93/722 parallels the Quantikine HS standard curve. To convert sample values obtained with the Quantikine HS Human IL-10 kit to approximate NIBSC units use the equation below.

NIBSC (93/722) approximate value (U/mL) = 0.012 x Quantikine HS Human IL-10 value (pg/mL)

SAMPLE VALUES

Serum - Twenty-two serum samples from apparently healthy volunteers were evaluated for the presence of human IL-10 in this assay. Twenty samples measured below the low standard, 0.78 pg/mL. Two samples measured 1.23 pg/mL and 3.47 pg/mL. No medical histories were available for the donors used in this study.

EDTA Plasma - Thirty-four EDTA plasma samples from apparently healthy volunteers were evaluated for the presence of human IL-10 in this assay. All samples measured below the low standard, 0.78 pg/mL. No medical histories were available for the donors used in this study.

Citrate Plasma - Sixteen citrate plasma samples from apparently healthy volunteers were evaluated for the presence of human IL-10 in this assay. All samples measured below the low standard, 0.78 pg/mL. No medical histories were available for the donors used in this study.

SPECIFICITY

This assay recognizes natural and recombinant human IL-10.

The factors listed below were prepared at 50 ng/mL in Calibrator Diluent and assayed for cross-reactivity. Preparations of the following factors prepared at 50 ng/mL in a mid-range recombinant human IL-10 control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant human:

IL-10 Rα
IL-10 Rβ
IL-19
IL-20
IL-20 Rα
IL-22
IL-22 Rα1
IL-24
IL-26/AK155 dimer
IL-26/AK155 monomer

Recombinant mouse:

IL-10
IL-10 Rα
IL-10 Rβ
IL-19
IL-20
IL-20 Rα
IL-20 Rβ
IL-22 Rα1
IL-24

Recombinant rat:

IL-10
IL-22

Other recombinants:

equine IL-10
feline IL-10
guinea pig IL-10
porcine IL-10
viral CMV IL-10

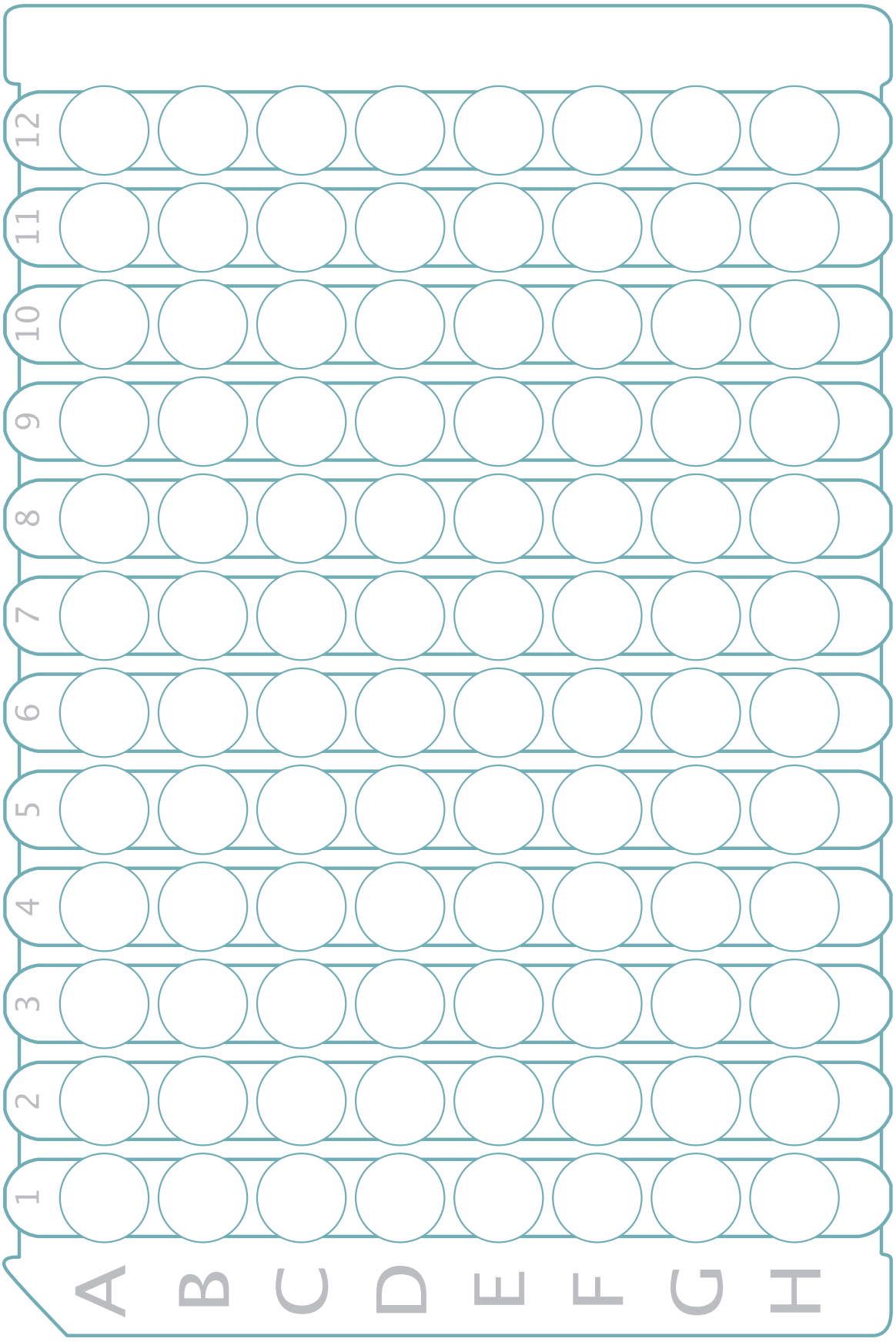
Recombinant canine IL-10 cross-reacts approximately 1% in this assay.

REFERENCES

1. O'Garra, A. and P. Vieira (2007) Nat. Rev. Immunol. **7**:425.
2. Pestka, S. *et al*. (2004) Annu. Rev. Immunol. **22**:929.
3. Vieira, P. *et al*. (1991) Proc. Natl. Acad. Sci. **88**:1172.
4. Windsor, W.T. *et al*. (1993) Biochemistry **32**:8807.
5. Syto, R. *et al*. (1998) Biochemistry **37**:16943.
6. Mathurin, P. *et al*. (2002) Am. J. Physiol. Gastrointest. Liver Physiol. **282**:G981.
7. Grewe, M. *et al*. (1995) J. Invest. Dermatol. **104**:3.
8. Szony, B.J. *et al*. (1999) Mol. Hum. Reprod. **5**:1059.
9. Kotenko, S.V. *et al*. (1997) EMBO J. **16**:5894.
10. Liu, Y. *et al*. (1994) J. Immunol. **152**:1821.
11. Carson, W.E. *et al*. (1995) Blood **85**:3577.
12. Denning, T.L. *et al*. (2000) Int. Immunol. **12**:133.
13. Ledeboer, A. *et al*. (2002) Eur. J. Neurosci. **16**:1175.
14. Lutfalla, G. *et al*. (1993) Genomics **16**:366.
15. Kotenko, S.V. *et al*. (2000) J. Biol. Chem. **276**:2725.
16. Hor, S. *et al*. (2004) J. Biol. Chem. **279**:33343.
17. Sheppard, P. *et al*. (2003) Nat. Immunol. **4**:63.
18. Fitzgerald, D.C. *et al*. (2007) Nat. Immunol. **8**:1372.
19. Wu, K. *et al*. (2007) Cell. Mol. Immunol. **4**:269.
20. Blackburn, S.D. and E.J. Wherry (2007)Trends Microbiol. **15**:143.
21. Mustea, A. *et al*. (2006) Anticancer Res. **26**:1715.
22. Sato, T. *et al*. (1996) Clin. Cancer Res. **2**:1383.
23. Bertorello, R. *et al*. (2004) Clin. Exp. Med. **4**:148.
24. Renaud, S.J. *et al*. (2007) Biol. Reprod. **76**:448.
25. Camejo, M.I. (2003) Arch. Androl. **49**:111.
26. Nath, S.K. *et al*. (2005) Hum. Genet. **118**:225.
27. Hurme, M. *et al*. (2003) J. Med. Virol. **70**:S48.
28. Howell, W.M. and M.J. Rose-Zerilli (2006) Fam. Cancer **5**:143.
29. Schosser, A. *et al*. (2007) Am. J. Med. Genet. B Neuropschiatr. Genet. **144**:347.

PLATE LAYOUT

Use this plate layout to record standards and samples assayed.



NOTES