Quantikine™ ELISA

Rat IL-2 Immunoassay

Catalog Number R2000
SR2000
PR2000

For the quantitative determination of rat Interleukin 2 (IL-2) concentrations in cell culture supernates, serum, and plasma.

This package insert must be read in its entirety before using this product.
For research use only. Not for use in diagnostic procedures.

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INTRODUCTION

Interleukin 2 (IL-2), also known as T cell growth factor (TCGF), is a 15-18 kDa variably glycosylated α-helical polypeptide that is a member of the Common gamma Chain (γ_c) cytokine family (1-4). It exists as a monomer and has a notably short half-life (< 30 minutes) (1). Rat IL-2 is synthesized as a 155 amino acid (aa) precursor that contains a 20 aa signal sequence plus a 135 aa mature region (5, 6). The mature region is α-helical in nature and contains one utilized O-linked glycosylation site at Thr3, plus three cysteines, two of which form an intrachain disulfide bond that is essential for activity (7). Mature rat IL-2 shares 66% and 73% aa identity with human and mouse IL-2, respectively. Although human IL-2 shares only approximately 60% aa identity with the highly polymorphic mouse IL-2, human IL-2 is known to be active on mouse IL-2 responsive cells. Cells reported to secrete IL-2 include γδ T cells (8), activated conventional $CD4^+$ and $CD8^+$ T cells (1, 9), neurons (10, 11), microglia (12), and hematopoietic stem cells (13).

The receptor for IL-2 (IL-2 R) is composed of three subunits, the 55 kDa CD25/IL-2 Rα chain, the 70 kDa IL-2 Rβ chain, and the 65 kDa γ_c (1, 3). IL-2 first binds to CD25; the binary complex then recruits IL-2 Rβ and γ_c to form the quaternary signaling complex (1, 14). In addition to IL-2, IL-2 Rβ is used by IL-15 in its quaternary signaling complex. The γc also serves as a signaling receptor for IL-4, -7, -9, -15, and -21 (1, 3).

In vitro studies have shown an important role for IL-2 in T cell activation and expansion. *In vivo*, IL-2 is critical for the development, maintenance and function of regulatory T cells (Treg) which provide protection against autoimmune disease. On the other hand, IL-2 can also promote autoimmune inflammation in target organs through its roles in regulating the expression of T cell trafficking genes and production of Th2 cytokines. Within the $CD8^+$ T cell subset, IL-2 is essential for optimal primary responses and differentiation into terminal effector cells. IL-2 also promotes the development of activated $CD8^+$ T cells into memory cells (1).

The Quantikine™ Rat IL-2 Immunoassay is a 4.5 hour solid phase ELISA designed to measure rat IL-2 levels in cell culture supernates, serum, and plasma. It contains *E. coli*-expressed recombinant rat IL-2 and antibodies raised against the recombinant factor. This immunoassay has been shown to quantitate the recombinant factor accurately. Results obtained using natural rat IL-2 showed dose response 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 natural rat IL-2.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A polyclonal antibody specific for rat IL-2 has been pre-coated onto a microplate. Standards, control, and samples are pipetted into the wells and any IL-2 present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for rat IL-2 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-2 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 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.
- For best results, pipette reagents and samples into the center of each well.
- It is recommended that the samples be pipetted within 15 minutes.
- 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 #	CATALOG # R2000	CATALOG # SR2000	DESCRIPTION	STORAGE OF OPENED/ RECONSTITUTED MATERIAL
Rat IL-2 Microplate	890593	2 plates	6 plates	96 well microplates (12 strips of 8 wells) coated with a polyclonal antibody specific for rat IL-2.	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.*
Rat IL-2 Standard	890595	3 vials	9 vials	Recombinant rat IL-2 in a buffered protein base with preservatives; lyophilized. *Refer to the vial label for reconstitution volume.*	Use a new standard and control for each assay. Discard after use.
Rat IL-2 Control	890596	3 vials	9 vials	Recombinant rat IL-2 in a buffered protein base with preservatives; lyophilized. The assay value of the control should be within the range specified on the label.	
Rat IL-2 Conjugate	890594	1 vial	3 vials	23 mL/vial of a polyclonal antibody specific for rat IL-2 conjugated to horseradish peroxidase with preservatives.	May be stored for up to 1 month at 2-8 °C.*
Assay Diluent RD1-21	895215	1 vial	3 vials	12 mL/vial of a buffered protein base with preservatives.	
Calibrator Diluent RD5-4	895435	2 vials	6 vials	21 mL/vial of a buffered protein base with preservatives.	
Wash Buffer Concentrate	895003	2 vials	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	3 vials	12 mL/vial of stabilized hydrogen peroxide.	
Color Reagent B	895001	1 vial	3 vials	12 mL/vial of stabilized chromogen (tetramethylbenzidine).	
Stop Solution	895174	1 vial	3 vials	23 mL/vial of a diluted hydrochloric acid solution.	
Plate Sealers	N/A	8 strips	24 strips	Adhesive strips.	

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

R2000 contains sufficient materials to run ELISAs on two 96 well plates.
SR2000 (SixPak) contains sufficient materials to run ELISAs on six 96 well plates.

This kit is also available in a PharmPak (R&D Systems®, Catalog # PR2000). Refer to the PharmPak Contents section for specific vial counts.

PHARMPAK CONTENTS

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

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

The reagents provided in this PharmPak are detailed below.

PART	PART #	QUANTITY
Rat IL-2 Microplate	890593	50 plates
Rat IL-2 Standard*	890595	50 vials
Rat IL-2 Control	890596	50 vials
Rat IL-2 Conjugate	890594	25 vials
Assay Diluent RD1-21	895215	25 vials
Calibrator Diluent RD5-4	895435	50 vials
Wash Buffer Concentrate	895126	9 bottles
Color Reagent A	895000	25 vials
Color Reagent B	895001	25 vials
Stop Solution	895174	25 vials
Plate Sealers	N/A	100 sheets

**If additional standard vials are needed, contact Technical Service at techsupport@bio-techne.com*

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
- 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 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.*
Grossly hemolyzed or lipemic samples may not be suitable for use with this assay.

SAMPLE PREPARATION

Serum and plasma samples require a 2-fold dilution prior to assay. A suggested 2-fold dilution is 75 µL of sample + 75 µL of Calibrator Diluent RD5-4.

REAGENT PREPARATION

Bring all reagents to room temperature before use.

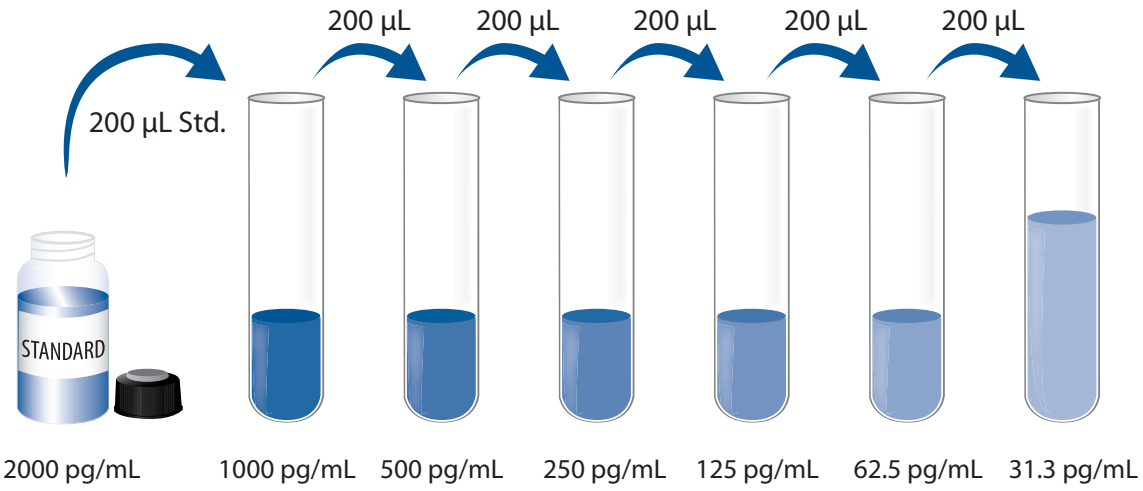
Rat IL-2 Control - Reconstitute the control with 1 mL of deionized or distilled water. Mix thoroughly. Assay the control undiluted.

Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. To prepare enough Wash Buffer for one plate, 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. 100 µL of the resultant mixture is required per well.

Rat IL-2 Standard - **Refer to the vial label for reconstitution volume.** Reconstitute the Rat IL-2 Standard with Calibrator Diluent RD5-4. Do not substitute other diluents. This reconstitution produces a stock solution of 2000 pg/mL. Allow the standard to sit for a minimum of 5 minutes with gentle mixing prior to making dilutions.

Pipette 200 µL of Calibrator Diluent RD5-4 into each tube. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The undiluted Rat IL-2 Standard (2000 pg/mL) serves as the high standard. Calibrator Diluent RD5-4 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, controls, and samples be assayed in duplicate.

1. Prepare reagents, standards, 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 RD1-21 to each well.
4. Add 50 µL of standard, control, or sample* per well. Mix by gently tapping the plate frame for 1 minute. Cover with the adhesive strip provided. Incubate for 2 hours at room temperature.
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 100 µL of Rat IL-2 Conjugate to each well. Cover with a new adhesive strip. Incubate for 2 hours at room temperature.
7. Repeat the aspiration/wash as in step 5.
8. Add 100 µL of Substrate Solution to each well. Incubate for 30 minutes at room temperature. **Protect from light.**
9. Add 100 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

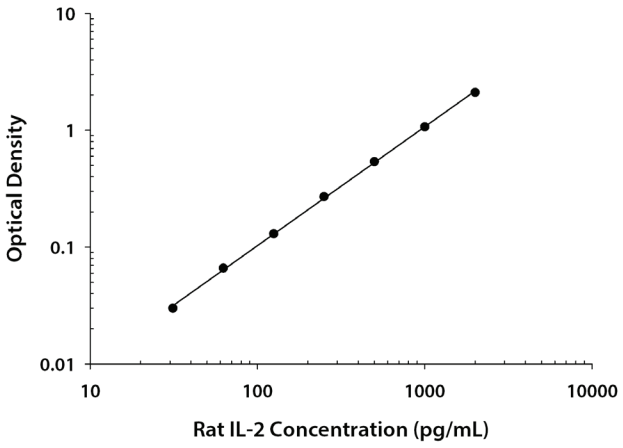
Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density (O.D.).

Create a standard curve by reducing the data using computer software capable of generating a log/log curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on a log/log graph. The data may be linearized by plotting the log of the rat IL-2 concentrations versus the log of the O.D. on a linear scale, and the best fit line can be determined by regression analysis.

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

TYPICAL DATA

This standard curve is provided for demonstration only. A standard curve should be generated for each set of samples assayed.



(pg/mL)	O.D.	Average	Corrected
0	0.034 0.034	0.034	—
31.3	0.065 0.063	0.064	0.030
62.5	0.105 0.096	0.100	0.066
125	0.166 0.163	0.164	0.130
250	0.308 0.300	0.304	0.270
500	0.574 0.571	0.572	0.538
1000	1.118 1.087	1.102	1.068
2000	2.161 2.117	2.139	2.105

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.

	Intra-Assay Precision			Inter-Assay Precision		
Sample	**1**	**2**	**3**	**1**	**2**	**3**
n	20	20	20	20	20	20
Mean (pg/mL)	130	478	1491	139	495	1550
Standard deviation	4.2	10.5	31.9	13.8	30.8	85.4
CV (%)	3.2	2.2	2.1	9.9	6.2	5.5

RECOVERY

The recovery of rat IL-2 spiked to three levels throughout the range of the assay in various matrices was evaluated.

Sample Type	Average % Recovery	Range
Cell culture supernates (n=8)	110	93-120%
Serum* (n=14)	96	83-114%
EDTA plasma* (n=4)	102	90-117%
Heparin plasma* (n=4)	100	92-116%

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

SENSITIVITY

The minimum detectable dose (MDD) of rat IL-2 is typically less than 15 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.

CALIBRATION

This immunoassay is calibrated against highly purified *E. coli*-expressed recombinant rat IL-2 produced at R&D Systems®.

LINEARITY

To assess the linearity of the assay, four or more samples containing and/or spiked with concentrations of rat IL-2 were diluted with calibrator diluent and then assayed.

Sample	Dilution	Observed (pg/mL)	Expected (pg/mL)	$\frac{\text{Observed}}{\text{Expected}} \times 100$
Cell culture supernates	Neat	1627	———	———
	1:2	851	814	105%
	1:4	413	407	101%
	1:8	210	204	103%
	1:16	101	102	99%
Serum*	Spiked	1057	———	———
	1:2	557	528	105%
	1:4	286	264	108%
	1:8	132	132	100%
	1:16	65	66	98%
EDTA plasma*	Spiked	876	———	———
	1:2	462	438	106%
	1:4	238	219	109%
	1:8	127	110	115%
	1:16	64	55	116%
Heparin plasma*	Spiked	1023	———	———
	1:2	561	512	110%
	1:4	279	256	109%
	1:8	131	128	102%
	1:16	68	64	106%

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

SAMPLE VALUES

Serum - Forty serum samples were evaluated for the presence of rat IL-2 in this assay. Thirty-nine samples measured below the lowest standard, 31.3 pg/mL. One sample measured 238 pg/mL.

Plasma - Twenty EDTA plasma samples and fourteen heparin plasma samples were evaluated for the presence of rat IL-2 in this assay. Most of the samples measured below the lowest standard, 31.3 pg/mL. Two EDTA plasma samples measured 80 pg/mL and 92 pg/mL, respectively. One heparin plasma sample measured 62 pg/mL.

Cell Culture Supernates - Rat splenocytes (1 x 10^7 cells/mL) were cultured for 3 days in RPMI plus 10% fetal bovine serum and stimulated with 5.0 µg/mL Concanavalin A. An aliquot of the cell culture supernate was removed, assayed for rat IL-2, and measured 14 ng/mL.

SPECIFICITY

This assay recognizes natural and recombinant rat IL-2.

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 rat IL-2 control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant rat:
CINC-1
GDNF
IFN-γ
IL-1α
IL-4
β-NGF
PDGF-BB
TNF-α

Recombinant mouse:
IL-2

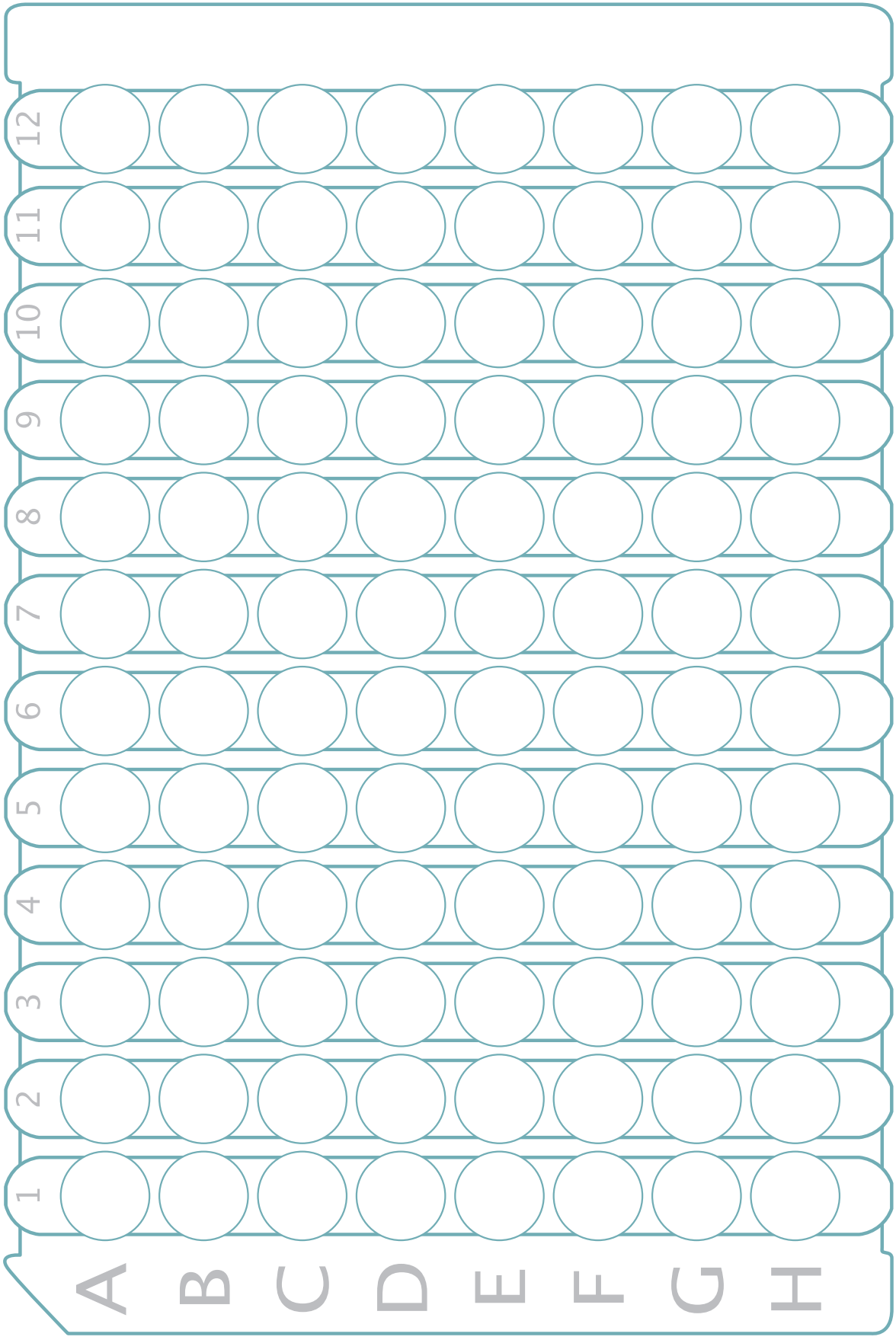
Recombinant human:
IL-2
IL-2 Rα
IL-2 Rβ

REFERENCES

1. Malek, T.R and I. Castro (2010) Immunity **33**:153.
2. Waldman, T.A. (2006) Nat. Rev. Immunol. **6**:595.
3. Malek, T.R. (2008) Annu. Rev. Immunol. **26**:453.
4. Conradt, H.S. *et al.* (1989) J. Biol. Chem. **264**:17368.
5. McKnight, A.J. *et al.* (1989) Immunogenetics **30**:145.
6. Rosenberg, S.A. *et al.* (1984) Science **223**:1412.
7. Smith, K.A. (1984) Annu. Rev. Immunol. **2**:319.
8. Tsukaguchi, K. *et al.* (1995) J. Immunol. **154**:1786.
9. Conlon, K. *et al.* (1995) Eur. J. Immunol. **25**:644.
10. Hwang, I.K. *et al.* (2006) Brain Res. **1106**:197.
11. Giestal de Araujo, E. *et al.* (2009) Ann. N.Y. Acad. Sci. **1153**:57.
12. Kowalski, J. *et al.* (2004) Pol. J. Pharmacol. **56**:563.
13. Eguizabal, C. *et al.* (2007) Int. J. Dev. Biol. **51**:731.
14. Stauber, D.J. *et al.* (2006) Proc. Natl. Acad. Sci. USA **103**:2788.

PLATE LAYOUT

Use this plate layout to record standards and samples assayed.



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