Quantikine™ ELISA

Rat IL-10 Immunoassay

Catalog Number R1000
SR1000
PR1000

For the quantitative determination of rat Interleukin 10 (IL-10) concentrations in cell culture supernates, serum, and EDTA 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-10 (IL-10), also known as cytokine synthesis inhibitory factor (CSIF), is the charter member of the IL-10 α-helical cytokine family that also includes IL-19, IL-20, IL-22, IL-24, and IL-26/AK155 (1-3). IL-10 is secreted by many activated hematopoietic cell types as well as hepatic stellate cells, keratinocytes, and placental cytotrophoblasts. Whereas human IL-10 is active on mouse cells, mouse IL-10 does not act on human cells (4, 5). Mature rat IL-10 shares 85% amino acid sequence identity with mouse IL-10 and 71% - 79% with bovine, canine, equine, feline, guinea pig, human, ovine, and porcine IL-10. It contains two intrachain disulfide bridges and is expressed as a 36 kDa noncovalently-associated homodimer (4, 6, 7).

IL-10 mediates its biological activities through a heteromeric receptor complex composed of the type II cytokine receptor subunits IL-10 Rα and IL-10 Rβ. IL-10 Rα is a 110 kDa transmembrane glycoprotein that is expressed on lymphocytes, NK cells, macrophages, monocytes, astrocytes, intestinal epithelial cells, cytotrophoblasts, and activated hepatic stellate cells (8-13), while the 75 kDa transmembrane IL-10 Rβ is widely expressed (14, 15). The IL-10 dimer binds to two IL-10 Rα chains, triggering recruitment of two IL-10 Rβ chains (14, 15). IL-10 Rβ does not bind IL-10 directly but is required for signal transduction. IL-10 Rβ also associates with IL-20 Rα, IL-22 Rα1, or IL-28 Rα to form the receptor complexes for IL-22, IL-26, IL-28, and IL-29 (16-18).

The involvement of IL-10 in immunoregulation includes both suppressive and stimulatory effects. It functions as an anti-inflammatory cytokine by inhibiting the expansion and activation of Th1 cells and Th17 cells (19-21), and by promoting the development of M2 macrophages (21). Its expression by immunosuppressive regulatory T cells (Treg) and regulatory B cells is important for Treg proliferation (19). Within a tumor microenvironment, however, IL-10 inhibits the expansion of Treg as well as myeloid-derived suppressor cells (22, 23). IL-10 induces the intratumoral accumulation and activation of CD8+ T cells (24, 25). IL-10 exerts protective effects including limiting tissue damage in arthritic inflammation (19) and promoting muscle regeneration after injury (21), but it also contributes to the persistence of viral infections (26). The levels of IL-10 have been found to be elevated in Sjogren's syndrome (saliva), primary CNS lymphoma (cerebrospinal fluid), and ovarian cancer (serum and ascites) (27-29). Its levels have been found to be decreased in the serum of patients with recurrent heart attacks or during preeclampsia and also in the seminal fluid of infertile men (30-32).

The Quantikine™ Rat IL-10 Immunoassay is a 4.5 hour solid phase ELISA designed to measure rat IL-10 levels in cell culture supernates, serum, and EDTA plasma. It contains *E. coli*-expressed recombinant rat IL-10 and antibodies raised against the recombinant factor. This immunoassay has been shown to quantitate the recombinant rat IL-10 accurately. Results obtained using natural rat IL-10 showed dose-response curves that were parallel to the standard curves obtained using the recombinant Quantikine kit standards. These results indicate that this kit can be used to determine relative mass values for natural rat IL-10.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for rat IL-10 has been pre-coated onto a microplate. Standards, control, 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 polyclonal antibody specific for rat 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. 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-10 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.
- It is important that the calibrator diluent selected for the standard curve be consistent with the samples being assayed.
- If samples generate values higher than the highest standard, 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.
- 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 #	CATALOG # R1000	CATALOG # SR1000	DESCRIPTION	STORAGE OF OPENED/ RECONSTITUTED MATERIAL
Rat IL-10 Microplate	890741	1 plate	6 plates	96 well microplate (12 strips of 8 wells) coated with a monoclonal antibody specific for rat IL-10.	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-10 Standard	890742	2 vials	12 vials	Recombinant rat IL-10 in a buffered protein base with preservatives; lyophilized. *Refer to the vial label for reconstitution volume.*	Discard within 8 hours of reconstitution. Use a new Standard and control for each assay.
Rat IL-10 Control	890744	2 vials	12 vials	Recombinant rat IL-10 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-10 Conjugate	890743	1 vial	6 vials	12 mL/vial of a polyclonal antibody against rat IL-10 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 RD5-3	895436	1 vial	3 vials	21 mL/vial of a buffered protein base with preservatives. *For cell culture supernate samples.*	
Calibrator Diluent RD5T	895175	1 vial	3 vials	21 mL/vial of a buffered protein base with preservatives. *For serum/plasma samples.*	
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	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	4 strips	24 strips	Adhesive strips.	

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

R1000 contains sufficient materials to run an ELISA on one 96 well plate.
SR1000 (SixPak) contains sufficient materials to run ELISAs on six 96 well plates.

This kit is also available in a PharmPak (R&D Systems®, Catalog # PR1000). 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®, Catalog # WA126).*

The reagents provided in this PharmPak are detailed below.

PART	PART #	QUANTITY
Rat IL-10 Microplate	890741	50 plates
Rat IL-10 Standard*	890742	50 vials
Rat IL-10 Control	890744	50 vials
Rat IL-10 Conjugate	890743	50 vials
Assay Diluent RD1-21	895215	25 vials
Calibrator Diluent RD5-3	895436	25 vials
or		
Calibrator Diluent RD5T	895175	25 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
- **Polypropylene** test tubes for dilution of standards

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 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: *Heparin and citrate plasma samples have not been validated for use in this assay. Grossly hemolyzed or lipemic samples may not be suitable for use with this assay.*

REAGENT PREPARATION

Bring all reagents to room temperature before use.

Rat IL-10 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. 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-10 Standard - **Refer to the vial label for reconstitution volume.** Reconstitute the Rat IL-10 Standard with Calibrator Diluent RD5-3 (*for cell culture supernate samples*) or Calibrator Diluent RD5T (*for serum/plasma samples*). 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.

Use polypropylene tubes. Pipette 200 µL of the Calibrator Diluent RD5-3 (*for cell culture supernate samples*) or Calibrator Diluent RD5T (*for serum/plasma samples*) 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-10 Standard (2000 pg/mL) serves as the high standard. The appropriate calibrator diluent serves as the zero standard (0 pg/mL).

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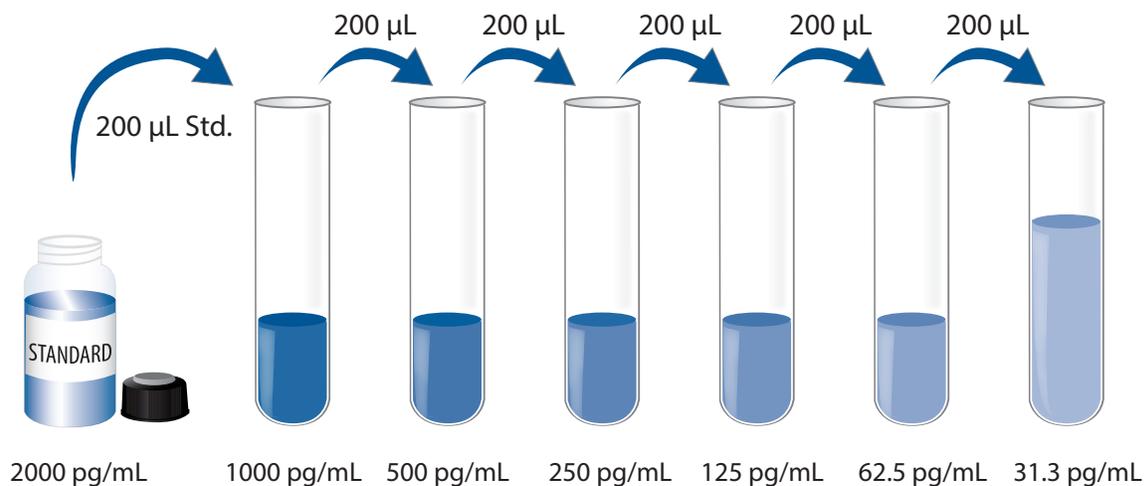
ASSAY PROCEDURE

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, samples, and standard dilutions 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. 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 100 µL of Rat IL-10 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.

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 rat 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

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

CELL CULTURE SUPERNATE ASSAY
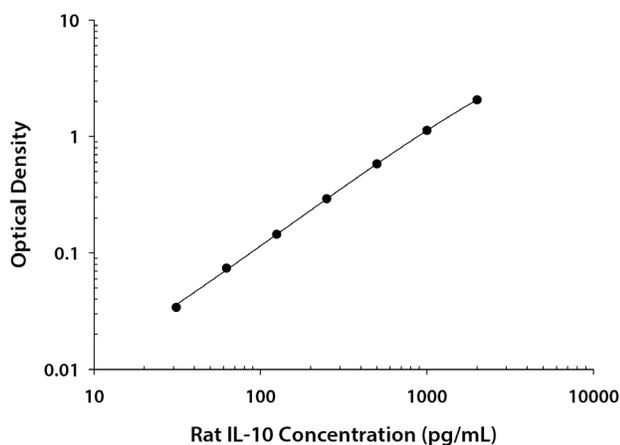
(pg/mL)	O.D.	Average	Corrected
0	0.067 0.065	0.066	—
31.3	0.099 0.100	0.100	0.034
62.5	0.139 0.141	0.140	0.074
125	0.211 0.208	0.210	0.144
250	0.357 0.358	0.358	0.292
500	0.654 0.637	0.646	0.580
1000	1.194 1.194	1.194	1.128
2000	2.055 2.210	2.132	2.066

SERUM/PLASMA ASSAY
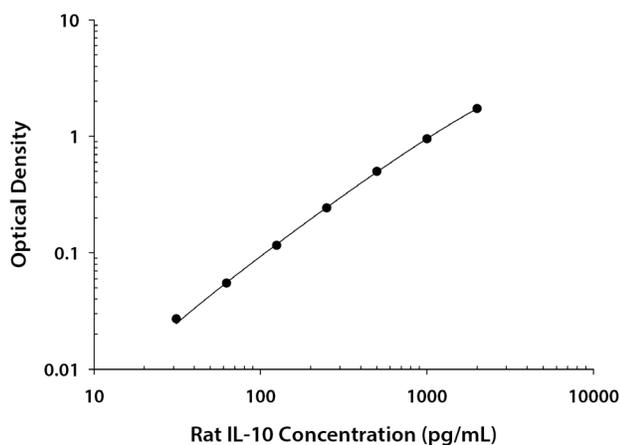
(pg/mL)	O.D.	Average	Corrected
0	0.049 0.053	0.051	—
31.3	0.078 0.078	0.078	0.027
62.5	0.107 0.105	0.106	0.055
125	0.169 0.165	0.167	0.116
250	0.295 0.293	0.294	0.243
500	0.553 0.546	0.550	0.499
1000	0.994 1.015	1.004	0.953
2000	1.767 1.798	1.782	1.731

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.

CELL CULTURE SUPERNATE ASSAY

	Intra-Assay Precision			Inter-Assay Precision		
Sample	1	2	3	1	2	3
n	20	20	20	20	20	20
Mean (pg/mL)	103	218	582	114	236	598
Standard deviation	5.7	9.5	17.2	10.8	16.4	42.6
CV (%)	5.5	4.4	3.0	9.5	6.9	7.1

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)	138	270	660	132	270	682
Standard deviation	6.3	9.0	16.1	13.1	19.7	59.8
CV (%)	4.6	3.3	2.4	9.9	7.3	8.8

RECOVERY

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

Sample Type	Average % Recovery	Range
Cell culture supernates (n=5)	106	96-118%
Serum (n=6)	94	82-112%
EDTA plasma (n=6)	100	88-115%

SENSITIVITY

The minimum detectable dose (MDD) of rat IL-10 is typically less than 10 pg/mL.

The MDD was determined by adding two standard deviations to the mean O.D. value of 20 zero standard replicates and calculating the corresponding concentration.

CALIBRATION

This immunoassay is calibrated against highly purified *E. coli*-expressed recombinant rat IL-10 produced at R&D Systems®.

LINEARITY

To assess the linearity of the assay, five or more samples spiked with various concentrations of rat IL-10 in each matrix were diluted with the appropriate calibrator diluent and then assayed. Results from typical sample dilutions are shown.

Sample	Dilution	Observed (pg/mL)	Expected (pg/mL)	$\frac{Observed}{Expected}$ x 100
Cell culture supernates	Spiked	1378	——	——
	1:2	697	689	101
	1:4	342	344	99
	1:8	166	172	97
	1:16	81	86	94
Serum	Spiked	1034	——	——
	1:2	506	517	98
	1:4	267	258	103
	1:8	131	129	102
	1:16	69	64	108
EDTA plasma	Spiked	1177	——	——
	1:2	616	588	105
	1:4	312	294	106
	1:8	147	147	100
	1:16	70	74	95

SAMPLE VALUES

Serum - Twenty samples were evaluated for detectable levels of rat IL-10 in this assay. Nineteen samples measured below the lowest standard, 31.3 pg/mL. One sample measured 34.8 pg/mL.

EDTA Plasma - Twenty samples were evaluated for detectable levels of rat IL-10 in this assay. Fourteen samples measured below the lowest standard, 31.3 pg/mL. Six samples measured 32.3-59.9 pg/mL.

Cell Culture Supernates - Rat splenocytes (1 x 10^7 cells/mL) were cultured for 2 days in DMEM supplemented with 10% fetal calf serum and stimulated with 5 µg/mL Conconavalin A. An aliquot of the cell culture supernate was removed, assayed for rat IL-10, and measured 340 pg/mL.

SPECIFICITY

This assay recognizes natural and recombinant rat 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 rat IL-10 control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant rat:

CINC-1	IL-4
GDNF	IL-6
GM-CSF	IL-18
IFN-γ	β-NGF
IL-1α	PDGF-BB
IL-1β	TNF-α
IL-2	

Recombinant mouse:
IL-10 R

Recombinant human:
IL-10
IL-10 R

Recombinant mouse IL-10 cross-reacts approximately 1.8% in this assay.

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PLATE LAYOUT

Use this plate layout to record standards and samples assayed.

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