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R&D SYSTEMS

Quantikine™ QuicKit™ ELISA

Human CD25/IL-2 Rα Immunoassay

Catalog Number QK0223
SK0223
PK0223

For the quantitative determination of human Interleukin 2 Receptor alpha (IL-2 Rα) concentrations in cell culture supernates, cell lysates, 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

SECTION PAGE

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INTRODUCTION

The biological activities of IL-2 are mediated by its binding to a multi-molecular cellular receptor complex. For several years the receptor was thought to consist of two glycoprotein chains, an alpha chain (IL-2 Rα) and a beta chain (IL-2 Rβ) (1-3), which acted together to form a high affinity receptor that transduced the IL-2 signal. IL-2 Rα (also known as Tac antigen and as CD25) is a 55 kDa transmembrane glycoprotein composed of 351 amino acids with only 13 located on the cytoplasmic side of the membrane (4-6). The second chain of the complex was cloned in 1989 (7), and is a transmembrane glycoprotein of 575 amino acids (75 kDa), 286 of which are located cytoplasmically and clearly participate in signal transduction (8, 9). Eventually it was discovered that a third chain, IL-2 Rγ, was necessary for high affinity binding, ligand internalization, and signalling. Constitutively expressed on many lymphoid cells, it had been overlooked partly because it has no affinity for IL-2 except when IL-2 Rβ is present (7, 10, 11). When cloned, the gene was found to code for a 64 kDa transmembrane protein of 347 amino acids, 84 of which are cytoplasmic (12). Both IL-2 Rβ and IL-2 Rγ are members of the hematopoietin receptor superfamily, whereas IL-2 Rα is related only to the IL-15 Rα chain (13-15).

A model of the IL-2 receptor complex (3, 9, 16-21) would describe the high affinity receptor as an αβγ trimer, in which all three chains are in contact with the ligand. Alone, IL-2 Rα binds IL-2 with low affinity, but is unable to transduce a signal. The αβ combination will bind IL-2 with intermediate affinity, but still will not transduce a signal. A βγ complex has intermediate affinity and is capable of signalling if the IL-2 concentration is relatively high. Regardless of many subtleties that determine the affinity of the ligand for the extracellular portions of the receptor components (22-24), signalling will ensue if the β and γ cytoplasmic domains are brought into close proximity (25-27).

A soluble form of IL-2 Rα appears in serum, concomitant with its increased expression on cells (18, 28, 29). There are reports of a soluble form of IL-2 Rβ as well (28, 30). The function of the soluble IL-2 Rα is unclear, since it would be expected to be a poor inhibitor of IL-2 because of its low binding affinity. In any case, increased levels of the soluble IL-2 Rα in biological fluids reportedly correlate with increased T and B cell activation and immune system activation. Results of a number of studies suggest a correlation of levels of IL-2 Rα in serum with the onset of rejection episodes in allograft recipients (18, 31-33), with activity of autoimmune diseases such as rheumatoid arthritis and systemic lupus erythematosis (SLE) (34) and with the course of some leukemias and lymphomas (35-40).

INTRODUCTION *CONTINUED*

The Quantikine™ QuicKit™ Human CD25/IL-2 Rα Immunoassay is a one-step, 80-minute solid phase ELISA designed to measure human IL-2 Rα levels in cell culture supernates, serum, plasma, and cell lysates. It contains recombinant human IL-2 Rα and antibodies raised against the recombinant protein. Results obtained for naturally occurring human IL-2 Rα showed linear curves that were parallel to the standard curves obtained using the recombinant QuicKit standards. These results indicate that this kit can be used to determine relative mass values for natural IL-2 Rα.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. An anti-tag antibody has been pre-coated onto a microplate. Standards and samples are pipetted into the wells followed by an antibody cocktail. The antibody cocktail consists of an affinity tag labeled monoclonal capture antibody and an enzyme-linked monoclonal detection antibody, specific for human IL-2 Rα. After washing away any unbound substances, a substrate solution is added to the wells and color develops in proportion to the amount of IL-2 Rα bound. 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 from other lots or sources.
- If samples generate values higher than the highest standard, dilute the samples with the 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™ QuicKit™ 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.
- Ensure reagent addition to plate wells is uninterrupted.
- To ensure accurate results, proper adhesion of the plate sealer during the incubation step is necessary.
- 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.
- 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. Wells that are green in color indicate that the Stop Solution has not mixed thoroughly with the Substrate Solution.

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.

Wear protective gloves, clothing, eye, and face protection. Wash hands thoroughly after handling. Refer to the SDS on our website prior to use.

MATERIALS PROVIDED & STORAGE CONDITIONS

Store the unopened kit at 2-8 °C. Do not use past kit expiration date.

PART	PART #	CATALOG # QK0223	CATALOG # SK0223	DESCRIPTION	STORAGE OF OPENED/ RECONSTITUTED MATERIAL
QuicKit™ Coated Microplate	899063	1 plate	6 plates	96 well polystyrene microplate (12 strips of 8 wells) coated with an anti-tag monoclonal antibody.	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.*
Human IL-2 Rα Standard	899579	2 vials	12 vials	Recombinant human IL-2 Rα in a buffered protein base with preservatives; lyophilized. *Refer to the vial label for the reconstitution volume.*	Use a freshly reconstituted standard for each assay. Discard after use.
Human IL-2 Rα Capture Ab Concentrate	899577	1 vial	6 vials	Lyophilized tagged monoclonal antibody specific for human IL-2 Rα.	May be stored for up to 1 month at 2-8 °C.*
Human IL-2 Rα Detection Ab Concentrate	899578	1 vial	6 vials	400 µL of a monoclonal antibody specific for human IL-2 Rα conjugated to horseradish peroxidase with preservatives.	
Assay Diluent RD1-55	895066	1 vial	6 vials	12 mL of a buffered protein base with preservatives.	
Calibrator Diluent RD5Z	895206	1 vial	6 vials	21 mL of a buffered protein base with preservatives.	
Wash Buffer Concentrate	895003	1 vial	6 vials	21 mL of a 25-fold concentrated solution of buffered surfactant with preservative. *May turn yellow over time.*	
TMB ELISA Substrate	642736	1 vial	6 vials	12 mL of a TMB ELISA substrate.	
ELISA Stop Solution	642827	1 vial	6 vials	12 mL of an acid solution.	
Plate Sealers	N/A	4 strips	8 strips	Adhesive strips.	

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

QK0223 contains sufficient materials to run an ELISA on one 96 well plate.
SK0223 (SixPak) contains sufficient materials to run ELISAs on six 96 well plates.

This kit is also available in a PharmPak (R&D Systems®, Catalog # PK0223). Refer to the PharmPak Contents section for specific vial counts.

PHARMPAK CONTENTS

Each PharmPak has enough reagents to assay 50 microplates (96 wells/plate). Although the inserts are the same as those for the single kit inserts, there are minor differences related to the number of reagents and their container sizes.

- 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
QuicKit™ Coated Microplate	899063	50 plates
Human IL-2 Rα Standard*	899579	25 vials
Human IL-2 Rα Capture Ab Concentrate	899577	50 vials
Human IL-2 Rα Detection Ab Concentrate	899578	50 vials
Assay Diluent RD1-55	895066	50 vials
Calibrator Diluent RD5Z	895206	50 vials
Wash Buffer Concentrate	895126	9 bottles
TMB ELISA Substrate	642736	50 vials
ELISA Stop Solution	642827	50 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
- Horizontal orbital microplate shaker (0.12" orbit) capable of maintaining a speed of 500 ± 50 rpm
- Human IL-2 Rα Controls (optional; Catalog # QC322)

For cell lysates samples (optional):

- Lysis Buffer 17 (R&D Systems®, Catalog # 895943)

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

Cell Lysates - Cells must be lysed prior to assay as directed in the Sample Values section.

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 heparin 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: *Citrate plasma has not been validated for use in this assay.*

SAMPLE PREPARATION

Cell culture supernates samples require a 4-fold dilution due to endogenous levels. A suggested 4-fold dilution is 50 µL of sample + 150 µL of Calibrator Diluent RD5Z.

Serum and plasma samples require a 4-fold dilution due to matrix effect. A suggested 4-fold dilution is 50 µL of sample + 150 µL of Calibrator Diluent RD5Z.

For cell lysate samples, quantitation of sample protein concentration using a total protein assay is recommended. The suggested range for total cell lysate protein added is 1-5 µg/well.

Multiple dilutions are recommended for unknown samples.

REAGENT PREPARATION

Bring all reagents to room temperature before use.

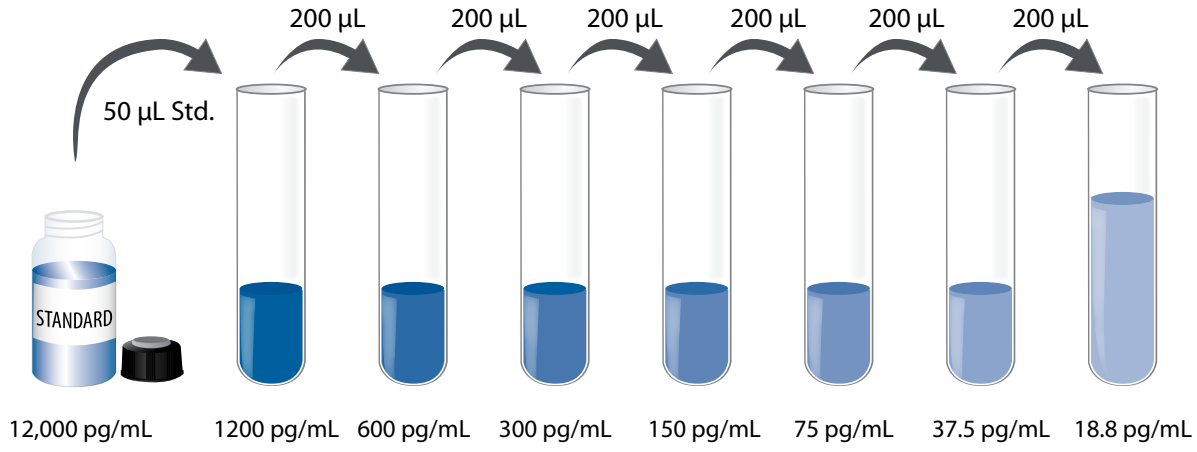
Human IL-2 Rα Capture Ab Concentrate - **Refer to the vial label for reconstitution volume.** Reconstitute the Human IL-2 Rα Capture Ab Concentrate with Assay Diluent RD1-55. This reconstitution produces a 20X Capture Antibody stock. Allow the capture antibody to sit for a minimum of 5 minutes with gentle agitation prior to diluting. Once reconstituted, the 20X capture antibody stock can be stored for 4 weeks at 2-8 °C.

Antibody Cocktail - Dilute the reconstituted Capture Ab stock and the Detection Ab Concentrate 20-fold in Assay Diluent RD1-55. For a full plate, add 300 µL of reconstituted Human IL-2 Rα Capture Ab stock and 300 µL of Human IL-2 Rα Detection Ab Concentrate to 5.4 mL of Assay Diluent RD1-55 to get 6 mL of Human IL-2 Rα Antibody Cocktail.

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.

Human IL-2 Rα Standard - **Refer to vial label for reconstitution volume.** Reconstitute the Human IL-2 Rα Standard with distilled or deionized water. This reconstitution produces a stock solution of 12,000 pg/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions.

Pipette 450 µL of Calibrator Diluent RD5Z into the 1200 pg/mL tube. Pipette 200 µL into the remaining tubes. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The 1200 pg/mL standard serves as the high standard. Calibrator Diluent RD5Z 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 all reagents, samples, 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 standard, control, or sample* per well. A plate layout is provided to record standards and samples assayed.
4. Add 50 µL Antibody Cocktail to each well. Cover with the adhesive strip provided. Incubate for 1 hour at room temperature on a horizontal orbital microplate shaker (0.12″ orbit) set at 500 ± 50 rpm.
5. Aspirate each well and wash, repeating the process twice for a total of three 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 TMB ELISA Substrate solution to each well. Incubate for 20 minutes at room temperature **on the benchtop. Protect from light.**
7. Add 100 µL of ELISA Stop Solution to each well. The color in the wells should change from blue to yellow. If the color in the wells is green or the color change does not appear uniform, gently tap the plate to ensure thorough mixing.
8. 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 require dilution. See Sample Preparation section.

CALCULATION OF RESULTS

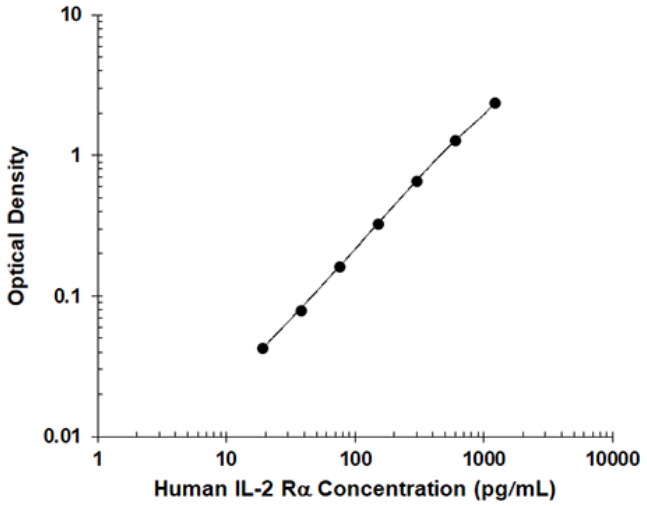
Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density (O.D.).

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 human IL-2 Rα 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

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	0.023 0.028	0.026	—
18.8	0.067 0.070	0.069	0.043
37.5	0.102 0.110	0.106	0.080
75.0	0.183 0.192	0.188	0.162
150	0.350 0.359	0.355	0.329
300	0.688 0.695	0.692	0.666
600	1.304 1.312	1.308	1.282
1200	2.349 2.436	2.393	2.367

PRECISION

Intra-Assay Precision (Precision within an assay)
Two samples of known concentration were tested twenty times on one plate to assess intra-assay precision.

Inter-Assay Precision (Precision between assays)
Two samples of known concentration were tested in ten separate assays to assess inter-assay precision. Assays were performed by at least four technicians.

	Intra-Assay Precision		Inter-Assay Precision	
Sample	1	2	1	2
n	20	20	10	10
Mean (pg/mL)	149	892	154	904
Standard deviation	3.37	24.1	11.5	46.7
CV (%)	2.3	2.7	7.5	5.2

RECOVERY

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

Sample Type	Average % Recovery	Range
Cell culture media (n=2)	100	93-105%
Serum (n=2)	91	87-100%
EDTA plasma (n=2)	93	83-97%
Heparin plasma (n=2)	92	84-97%
Lysis buffer (n=1)	101	96-106%

SENSITIVITY

Fourteen assays were evaluated and the minimum detectable dose (MDD) of human IL-2 Rα ranged from 2.31-6.94 pg/mL. The mean MDD was 4.40 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 human IL-2 Rα were diluted with calibrator diluent to produce samples with values within the dynamic range of the assay.

		Cell culture supernates (n=2)	Serum (n= 2)	EDTA plasma (n= 2)	Heparin plasma (n= 2)	Cell lysates (n= 2)
1:2	Average % of Expected	101	100	99	100	100
	Range (%)	100-102	98-102	99-99	100-100	98-101
1:4	Average % of Expected	101	102	99	98	99
	Range (%)	99-103	97-107	98-99	98-98	96-101
1:8	Average % of Expected	99	98	96	98	89
	Range (%)	97-101	93-103	93-99	91-104	88-90
1:16	Average % of Expected	95	92	91	93	93
	Range (%)	94-96	85-99	84-97	83-103	93-93

CALIBRATION

This immunoassay is calibrated against a highly purified recombinant human IL-2 Rα produced at R&D Systems®.

The NIBSC Interleukin-2 Soluble Receptor Non-WHO Reference Material 97/600 (Human rDNA derived) was evaluated in this kit. The dose response curve in this reference material parallels the Quantikine™ QuicKit Standard Curve. To convert sample values obtained with the Quantikine™ QuicKit Human IL-2 Rα Kit to approximate NIBSC 97/600 International Units, use the equation below.

NIBSC (97/600) approximate value (IU/mL) = 0.0363 x Quantikine™ QuicKit Human CD25/IL-2 Rα value (pg/mL)

Based on data generated from March 2025.

SAMPLE VALUES

Serum/Plasma - Samples from apparently healthy volunteers were evaluated for the presence of human IL-2 Rα in this assay. No medical histories were available for the donors used in this study.

Sample Type	Mean (pg/mL)	Range (pg/mL)	Standard Deviation (pg/mL)
Serum (n=10)	1670	637-4112	1018
EDTA plasma (n=10)	1624	789-4226	978
Heparin plasma (n=10)	1528	797-4008	922

Cell Culture Supernate/Cell lysates - Human peripheral blood mononuclear cells (PBMCs) were seeded at 1 x 10^6/mL and cultured in RPMI 1640 supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin sulfate. PBMCs were treated with 10 µg/mL PHA for 48 hours prior to collecting cell culture supernates. Supernates were then centrifuged to remove cells/debris, aliquoted, and assayed for levels of human IL-2 Rα. For the cell lysates, cells were washed with PBS before solubilizing in Lysis Buffer 17 (Catalog # 895943) with protease inhibitors, using 3-5X the cell pellet volume, and assayed for levels of human IL-2 Rα.

Sample Type	pg/mL
CCS Sample 1	4575
CCS Sample 2	7504
Cell Lysate Sample 1	402
Cell Lysate Sample 2	273

SPECIFICITY

This assay recognizes natural and recombinant human IL-2 Rα.

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 low level recombinant human IL-2 Rα control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant human:

IL-2
IL-2 Rβ
IL-2 Rγ

Recombinant mouse:

canine IL-2 Rα
equine IL-2 Rα
mouse IL-2 Rα
rat IL-2 Rα

Recombinant cynomolgus monkey IL-2 Rα interferes at concentrations > 25 ng/mL but does not cross-react in this assay.

Daclizumab interferes at concentrations > 10 ng/mL but does not cross-react in this assay.

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PLATE LAYOUT

Use this plate layout to record standards and samples assayed.