ELISA kits available from ADI (see details at the web site)

200-800-AVG **Avastin/Bevacizumab (Anti-VEGF) ELISA Kit for human, 96 tests**
200-810-ADG **Human Anti-Avastin/Bevacizumab IgG (anti-drug IgG) ELISA Kit for human, 96 tests**
200-820-VEF **Human VEGF ELISA Kit, 96 tests**
200-830-VEM **Mouse VEGF ELISA Kit, 96 tests**
200-840-VER **Rat VEGF ELISA Kit, 96 tests**
200-850-FLT **Human VEGFR1/FLT1 ELISA Kit, 96 tests**
200-860-KDR **Human VEGFR2/KDR ELISA Kit, 96 tests**
200-870-ID24 **Avastin/Bevacizumab identification/Counterfeit detection ELISA Kit, 24 tests**

#0010 Human Leptin
#200-120-AGH Human globular Adiponectin (gAcrp30)
#0700 Human Sex Hormone Binding Glob (SHBG)
#0900 Human IGF-Binding Protein 1 (IGFBP1)
#1000 Human C-Reactive Protein (CRP)
#100-110-RSH Human Resistin /FIZZ3
#100-140-ADH Human Adiponectin (Acrp30)
#100-160-ANH Human Angiogenin
#100-180-APH Human Angiopoietin-2 (Ang-2)
#100-190-B7H Human Bone Morphogenic Protein 7 (BMP-7)
#1190 Human Serum Albumin **#1200** Human Albumin (Urinary)
#1750 Human IgG (total) **#1760** Human IgM
#1800 Human IgE **#1810** Human Ferritin
#1210 Human Transferrin (Tf) **#0020** Beta-2 microglobulin
#1600 Human Growth Hormone (GH)

#0060 Human Pancreatic Colorectal cancer (CA-242)
#1820 Human Ovarian Cancer (CA125) **#1830** Human CA153
#1840 Human Pancreatic & GI Cancer (CA199)
#1310 Human Pancreatic Lipase
#1400 Human Prostatic Acid Phosphatase (PAP)
#1500 Human Prostate Specific Antigen (PSA) **#1510** free PSA (fPSA)
#0500 Human Alpha Fetoprotein (AFP)
#0050 Human Neuron Specific Enolase (NSE)

#0030 Human Insulin **#0040** Human C-peptide
#0100 Human Luteinizing Hormone (LH)
#0200 Human Follicle Stimulating Hormone (FSH)
#0300 Human Prolactin (PRL)
#0400 Human Chorionic Gonadotropin (HCG) #**0410** HCG-free beta

#0600 Human Thyroid Stimulating Hormone (TSH)
#1100 Human Total Thyroxine (T4) **#1110** Human Free T4 (fT4)
#1650 Human free triiodothyronine (fT3) **#1700** Human T3 (total)

#3000 Human Rheumatoid Factors IgM (RF)
#3100 Human anti-dsDNA

Instruction Manual No. M-210-345-I2R

Mouse IL-2 receptor alpha, soluble (IL2RsA/CD25) ELISA KIT

Cat # 210-345-I2R, 96 Tests

For Quantitative Determination of IL-2RsA/CD25 In Mouse Serum, plasma or cell culture medium

For In Vitro Research Use Only (RUO)

ALPHA DIAGNOSTIC INTERNATIONAL

6203 Woodlake Center Drive • San Antonio• Texas 78244 • USA.

Phone (210) 561-9515 • Fax (210) 561-9544

Toll Free (800) 786-5777

Email: service@4adi.com

Web Site: www.4adi.com

DRAFT MANUAL: PLEASE CONSULT THE MANUAL SUPPLIED WITH THE KIT FOR ANY LOT SPECIFIC CHNAGES.

Mouse IL-2R ELISA KIT # 210-345-I2R, 96 Tests

Kit Components	Cat #
Anti-mouse CD25 IgG coated strips, 96 wells, #210346-P	1 plate
Mouse IL-RA, Recombinant, **lyophilized** standard (10 ng), 2 vials; make more standards, #210347	2 vials
Biotinylated Anti-mouse IL-RA antibody (100x), 130 ul, # 210348	1 vial
Avidin-Biotin-Peroxidase complex (ABC) (100x) 130 ul, #210349	1 vial
Sample diluent buffer, 30 ml, #210345-SD	1 bottle
Antibody Diluent buffer, 12 ml , # 210345-AD	1 bottle
ABC Diluent buffer, 12 ml, #210345-ABC	1 bottle
TMB Substrate Solution, 10 ml, # 210345-TMB	1 bottle
Wash buffer (100X), 10 ml (dilute 1:100 with distilled water, **10 ml stock to 990 ml dH2O**) , #210345-WB	1 bottle
Stop solution, 10 ml, # 210345-SS	1 bottle
Complete Instruction Manual,# M-210-345-I2R	1

Intended use

Mouse interleukin-2 receptor (IL-2RsA) is a sandwich ELISA for quantitative detection of mouse IL-2RsA in cell culture supernates (culture medium), serum and plasma (heparin, EDTA, citrate). **For research use only (RUO)**, not for diagnosis, cure or prevention of the disease.

Introduction

CD25 is the alpha chain of the IL-2RsA receptor. T cells, activated B cells, some thymocytes, myeloid precursors, and oligodendrocytes that associates with CD122 to form a heterodimer that can act as a high-affinity receptor for IL-2. CD25 has been used as a marker to identify CD4+FoxP3+ regulatory T cells in mice. By in situ hybridization, the IL2R gene was mapped to 10p15-p14. Its soluble form, called *sIL-2R* may be elevated in these diseases and is occasionally used to track disease progression It is a type I transmembrane protein present on activated Gene Name IL2ra

Protein Name Interleukin-2 receptor subunit alpha

Protein Function Receptor for interleukin-2. The receptor is involved in the regulation of immune tolerance by controlling regulatory T cells (TREGs) activity. TREGs suppress the activation and expansion of autoreactive T-cells.
Uniprot ID IL-2ra-P01590 , Alternative Names Anakinra | Interleukin-2 receptor subunit alpha | IL-2 receptor subunit alpha | IL-2-RA | IL-2R subunit alpha | IL2-RA | P55 | CD25 antigen

Performance Characteristics

Range: 78-5000 pg/ml (mouse serum, plasma)

Sensitivity: < 5 pg/ml

Sensitivity, or Lower Limit of Detection (LLD), is the minimum level of target protein the ELISA assay can detect. We measure 20 blank wells and if the O.D. value is 2 standard deviations higher than the blanks' average O.D. the sample can be deemed positive

Specificity: Natural and recombinant mouse IL-2RsA

Cross-reactivity

No detectable cross-reactivity with other relevant proteins

Species cross-reactivity

The antibodies (anti-mouse IL-2RsA) used this kit has not been tested for potential crossreactive with IL-2RsA from species like monkey, mouse, and rat etc. ADI has separate ELISA kits for human and rat IL-2RsA.

Intra/Inter Assay Precision

	Intra-Assay Precision			Inter-Assay Precision		
Sample	1	2	3	1	2	3
n	16	16	16	24	24	24
Mean (pg/ml)	564	1244	2860	758	1654	3242
Standard deviation	32.7	75.9	157.3	54.6	134	217
CV(%)	5.8	6.1	5.5	7.2	8.1	6.7

Quick Summary of PLGF ELISA

1. Add **0.1 ml samples and standards and incubate the plate at 37°C for 90 min**. Remove the well content by aspiration or flicking over the waster container then blotting the wells over paper towels.
2. **Add 0.1 ml 1X biotinylated antibodies** and **incubate the plate at 37°C for 60 min**.
3. **Wash plate 5 times** with wash buffer (300 ul/wash).
4. **Add 0.1 1X ABC solution** and **incubate the plate at 37°C for 30 min**.
5. **Wash plate 5 times** with wash buffer
6. Add **90 ul TMB and incubate the plate at 37°C in dark for 20-25 min**.
7. Add **100 TMB stop solution** and **read at 450nm/630nm**.

References Steikesserer A (1992) Genomics 13, 654-657; Patterson D (1993) Genomics 15, 173-176;

8. **Add 90 µl of prepared TMB color** developing agent into each well and incubate plate at **37°C in dark for 20-25 min** (Note: blue color develops in 4 highest standards and positive samples. **T**he other wells show no obvious or intense color). Note: TMB incubation time may be varied 5-10 mins so as to get the highest standards A450 reading of 2.00-3.00 or within the linear reading range of most ELISA readers.

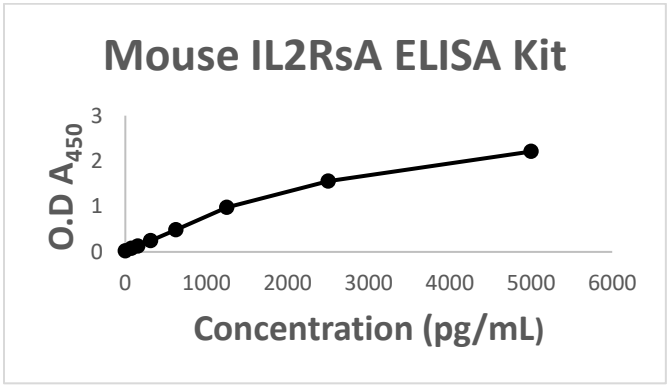
9. **Add 0.1 ml of prepared TMB stop solution into each well**. The color changes into yellow immediately. :

10. **Read the plates 450nm/630nm** (reference filter) in an ELISA reader within 30 min after adding the stop solution.

WORKSHEET OF TYPICAL ASSAY

Wells	Stds/samples	A450 Net Abs.
A1, A2	**blank** (0 pg/ml)	0.024
B1, B2	**Std. A** (78 pg/ml)	0.081
C1, C2	**Std. B** (156 pg/ml)	0.127
D1, D2	**Std. C** (313 pg/ml)	0.246
E1, E2	**Std. D** (625 pg/ml)	0.486
F1, F2	**Std. E** (1250 pg/ml)	0.982
G1, G2	**Std. F** (2500 pg/ml)	1.557
H1, H2	**Std. G** (5000 pg/ml)	2.210

NOTE: These data are for demonstration purpose only. A complete std. curve must be run in every assay to determine sample values.



A typical std curve (Plot linear graph Draw the best curve through the points. Do not use this for calculating sample values).

Calculations

Subtract the A450 values of blank or zero wells from all values. Plot A450 values of the standard on (Y) axis vs. concentration (X) on a semi-log scale. The sample PLGF concentration can be interpolated from the standard curve. **Note:** if the samples measured were diluted, multiply the dilution factor to the concentrations from interpolation to obtain the concentration before dilution.

In addition, placental sFlt-1 levels were significantly increased and PGF decreased in women with preeclampsia as compared to those with uncomplicated pregnancies. This suggests that placental concentrations of sFlt-1 and PGF mirror the maternal serum changes. This is consistent with the view that the placenta is the main source of sFlt-1 and PGF during pregnancy

PRINCIPLE OF THE TEST

Mouse IL-2RsA ELISA Kit was based on standard sandwich enzyme linked immune-sorbent assay technology. A monoclonal antibody from mouse specific for IL-2RsA has been precoated onto 96-well plates. Standards and test samples are added to the wells, a biotinylated detection polyclonal antibody from goat specific for IL2RsA is added subsequently and then followed by washing with PBS or TBS buffer. Avidin-Biotin-Peroxidase Complex was added and unbound conjugates were washed away with PBS or TBS buffer. HRP substrate TMB was used to visualize HRP enzymatic reaction. TMB was catalyzed by HRP to produce a blue color product that changed into yellow after adding acidic stop solution. The density of yellow is proportional to the mouse IL-2RsA amount of sample captured in plate.

MATERIALS AND EQUIPMENT REQUIRED

Adjustable micropipet (20-100 µl) and Multichannel pipet with disposable plastic tips. Reagent troughs, Plate washer (recommended) and ELISA plate Reader.

PRECAUTIONS

The IL-2RsA ELISA test is intended for *in vitro research* use only. The reagents contain thimerosal as preservative; necessary care should be taken when disposing solutions.. Applicable **MSDS**, if not already on file, **for** the following reagents can be obtained from ADI or the web site. TMB (substrate), H2SO4 (stop solution), and Prolcin-300 (0.1% v/v in standards, sample diluent and HRP-conjugates). All waste material should be properly disinfected before disposal. Avoid contact with the stop solution (1N sulfuric acid).

1. Sample Preparation and Storage

Store samples to be assayed within 24 hours at 2-8°C. For long-term storage, aliquot and freeze samples at -20°C. Avoid repeated freeze-thaw cycles.

- **Cell culture supernates**: Remove particulates by centrifugation, assay immediately or aliquot and store samples at -20°C.
- **Serum**: Allow the serum to clot in a serum separator tube (about 4 hours) at room temperature. Centrifuge at approximately 1000 X g for 15 min. Analyze the serum immediately or aliquot and store frozen at -20°C.
- **Plasma**: Collect plasma using heparin, EDTA or citrate as an anticoagulant. Centrifuge for 20 min at 2000 x g within 30 min of collection. Analyze immediately or aliquot and store frozen at -20°C.

2. Sample Dilution Guideline

The user needs to estimate the concentration of the target protein in the sample and select a proper dilution factor so that the diluted target protein concentration falls near the middle of the linear regime in the standard curve. Dilute the sample using the provided diluent buffer. The following is a guideline for sample dilution. Several trials may be necessary in practice. The sample must be well mixed with the diluents buffer.

- **High target protein** concentration (50-500ng/ml). The working dilution is 1:100. i.e. Add 1µl sample into 99 µl sample diluent buffer.
- **Medium target protein** concentration (5-50ng/ml). The working dilution is 1:10. i.e. Add 10µl sample into 90 µl sample diluent buffer.
- **Low target protein** concentration (78-5000 pg/ml). The working dilution is 1:2. i.e. Add 50 µl sample to 50 µl sample diluent buffer.
- **Very Low target protein** concentration (≤78 pg/ml). No dilution necessary, or the working dilution is 1:2.

Reconstitution of the mouse IL-RA standards:

IL-2RsA standard solution should be prepared no more than 2 hours prior to the experiment. Two tubes of IL-RA standard (10ng or 10,000 pg per tube) are included in each kit. Use one tube for each experiment. **Note:** The standard solutions are best used within 2 hours. The 10ng/ml standard solution should be stored at 4°C for up to 12 hours, or at -20°C for up to 48 hours. Avoid repeated freeze-thaw cycles.

Prepare stock standard of 10,000 pg/ml of mouse IL-RA (Stock): Add 1 ml sample diluent buffer into one tube, mix gently and leave the tube at room temp. for 10 min and. Do not vortex vigorously.

Stds	Sample diluent	Total volume	**Final Conc pg/ml**
0.5 ml of Stock (10,000 pg/ml)	0.5 ml	1 ml	Std H, 5000 pg/ml

Stds G-A are prepared by 2-fold serial dilutions

	Sample dilute	**Std Concn**
0.3 ml of Std H, 5000 pg/ml	0.30 ml	Std G 2500 pg/ml
0.3 ml of Std G, 2500 pg/ml	0. 30 ml	Std F, 1250 pg/ml
0.3 ml of Std F 1250 pg/ml	0. 30 ml	Std E, 625 pg/ml
0.3 ml of Std E, 625 pg/ml	0. 30 ml	Std D, 313 pg/ml
0.3 ml of Std D, 313 pg/ml	0. 30 ml	Std C, 156 pg/ml
0.3 ml of Std C, 156 pg/ml	0. 30 ml	Std B, 78 pg/ml
0.3 ml of Std B, 78 pg/ml	0. 30 ml	Std A, 15.6 pg/ml

Note: Gently mix the standard and diluent with the pipette before taking the standard for the next dilution.

1. Preparation of 1x-biotin anti-mouse IL-2RsA antibody solution:

The solution should be prepared no more than **2 hours** prior to the experiment.
Biotinylated anti-mouse IL-2R antibody should be diluted in 1:100 with the antibody diluent buffer and mixed thoroughly. (i.e. Add 10 µl Biotinylated anti-mouse IL-2R antibody to 990 µl antibody diluent buffer or 0.ml stock in 9.9 ml diluent). Prepare 1 ml for each strip or 10 ml for the full plate. Need 100 ul/well.

2. Preparation of 1X-Avidin-Biotin-Peroxidase Complex (ABC) solution:

3. **Avidin- Biotin-Peroxidase Complex (ABC**) should be diluted in **1:100 with the ABC dilution buffer** and mixed thoroughly. (i.e. Add 10 µl ABC to 990 µl ABC diluent buffer or 100 ul to 9.9 ml diluent.)

The solution should be prepared no more than 1 hour prior to the experiment. Need 0.1ml/well. Prepare 1 ml for 1 strip or 10 ml for full plate.

4. **Preparation of 1X wash buffer:** Dilute wash buffer (1:100) with distilled water (10 ml stock to 990 ml of distilled or deionized water). Store at 4oC.

STORAGE AND STABILITY

The microtiter well plate and all other reagents are stable at 2-8ºC until the expiration date printed on the label. The whole kit stability is usually 6 months from the date of shipping under appropriate storage conditions. Once opened/used standards are stable for two month at 2-8°C.

STORAGE AND STABILITY

The microtiter well plate and all other reagents are stable at 2-8ºC until the expiration date printed on the label. The whole kit stability is usually 6 months from the date of shipping under appropriate storage conditions. Once opened/used standards are stable for two month at 2-8°C. The unused portions of the standards should be frozen in suitable aliquots for long-term use. Repeated freezing and thawing is not recommended.

TEST PROCEDURE

Prepare standards, samples, and all reagents before using the kit. The ABC working solution and TMB color developing agent must be kept warm at 37°C for 30 min before use. When diluting samples and reagents, they must be mixed completely and evenly. Standard IL-RA detection curve should be prepared for each experiment. The user will decide sample dilution fold by crude estimation of IL-RA amount in samples. Remove required number of coated strips and arrange them on the plate. Store unused strips in the bag.

1. Add 0.1 ml per well of the (5000, 2500, 1250, 625, 313, 156, and 78 pg/ml mIL-2RsA) into the coated wells. Add 0.1ml of the sample diluent buffer into the control well (blank or Zero well). Add 0.1ml of each properly diluted sample of human cell culture supernates, serum or plasma(heparin, EDTA, citrate) to each empty well. **See "Sample Dilution Guideline" above for details.** We recommend that each mouse IL-2RsA standard solution and each sample is measured in duplicate.

2. Seal the plate with the cover and **incubate at 37°C for 90 min**.

3. Remove the cover, discard plate content, and blot the plate onto paper towels or other absorbent material. Do NOT let the wells completely dry at any time.

4. **Add 0.1ml of 1x biotinylated anti-mouse IL-RA antibody** working solution into each well and incubate the plate at **37°C for 60 min**.

5. **Wash plate 3 times** with 1x wash buffer (300 ul/well), each time let washing buffer stay in the wells for 1 min. Discard the washing buffer and blot the plate onto paper towels or other absorbent material. Repeat this process two additional times for a total of THREE washes. **Note**: For automated washing, aspirate all wells and wash THREE times with 300 ul wash buffer. Blot the plate onto paper towels or other absorbent material.)

6. **Add 0.1ml of prepared 1X ABC working solution** into each well and **incubate the plate at 37°C for 30 min**.

7. **Wash plate 5 times** as in step 5.

8. **Add 90 µl of prepared TMB color** developing agent into each well and incubate plate at **37°C in dark for 20-25 min** (Note: blue color develops in 4 highest standards and positive samples. **T**he other wells show no obvious or intense color). Note: TMB incubation time may be varied 5-10 mins so as to get the highest standards A450 reading of 2.00-3.00 or within the linear reading range of most ELISA readers.

9. **Add 0.1 ml of prepared TMB stop solution into each well**. The color changes into yellow immediately. :

10. **Read the plates 450nm/630nm** (reference filter) in an ELISA reader within 30 min after adding the stop solution.