Human IL-13 Rα2 ELISA Kit

For the quantitative determination of human interleukin 13 receptor α2 (IL-13 Rα2) concentrations in cell culture supernatant, serum and plasma

Catalogue Number: EL10039

96 tests

FOR LABORATORY RESEARCH USE ONLY
NOT FOR USE IN DIAGNOSTIC PROCEDURES

ANOGEN

2355 Derry Road East, Unit 23
Mississauga, Ontario
CANADA L5S 1V6
Tel: (905) 677-9221 or (877) 755-8324
Fax: (905) 677-0023

Email: info@anogen.ca ♦ Web Site: www.anogen.ca

S7.5 (04) IL-13 Rα2

TABLE OF CONTENTS

	Page
INTENDED USE	2
INTRUDUCTION	2
PRINCIPLE OF THE ASSAY	2
LIMITATION OF THE PROCEDURE	3
REAGENTS PROVIDED	4
MATERIALS REQUIRED BUT NOT SUPPLIED	5
PRECAUTIONS	5
SAMPLE PREPARATION	6
.....Collection, Handling and Storage	6
PREPARATION OF REAGENTS	6
ASSAY PROCEDURE	8
CALCULATION OF RESULTS	9
TYPICAL DATA	9
SENSITIVITY	10
SPECIFICITY	10
REFERENCES & CITATIONS	11

INTENDED USE

This Human Interleukin 13 Rα2 ELISA Kit is to be used for the *in vitro* quantitative determination of human interleukin 13 Rα2 concentrations in cell culture supernatant. This kit is intended FOR LABORATORY RESEARCH USE ONLY and not for use in diagnostic or therapeutic procedures.

INTRODUCTION

IL-13 receptor alpha 2, also known as CD213a2, is a subunit of IL-13 receptor complex. It is encoded by the gene locus Xq13.1-q28 on chromosome X. An important characteristic of this subunit is that it does not have a cytoplasmic domain. Even though, the IL-13 Ra2 binds to IL 13 with high affinity, the receptor appears not directly involved with a typical IL-13 stimulated signal transduction. Unlike IL-13Ra1, the binding of IL-13Ra2 to IL-13 is IL-4R independent and is not associated with Jaks and the phosphorylation and activation of Stats. There are evidences that soluble IL-13Ra2 may serve as decoy receptor to dampen the cell reaction to IL-13 stimulation.

IL-13Rα2 exists both on the cell membrane, and in soluble form. IL-13Rα2 is relatively rich in hemopoietic or hypervascular tissues, while expressed in low level or not expressed in other tissues. The gene was initially cloned from a kidney cell line and then found to be expressed in brain, spleen, liver, thymus, and also in the bone marrow.

The research on IL-13Ra2 has led to the discovery of association of abnormal IL-13Ra2 expression with a number of pathological conditions. The IL-13Ra2 gene polymorphism was found to be involved with susceptibility to Systemic Sclerosis (Brigitte Granel et al. 2006). The overexpression of *Il13ra2* gene has been found in brain tumors and ovarian cancer, making IL-13Ra2 one of the candidates as biomarker and target for immunotherapy. In the research by Mitomu Kioi et al in 2006, IL-13 cytotoxin was highly cytotoxic to the IGROV-1 ovarian cancer cell line in vitro, and it mediated significant antitumor activity against a xenografted tumor model. The result suggested that IL-13Ra2 could be a promising target for ovarian cancer therapy. Vaccine therapy of glioma was tested by vaccination using dendritic cells transfected with *Il13ra2* mRNA and this experiment induced strong anti-tumor effects in mice (Makoto Saka et al. 2010).

PRINCIPLE OF THE ASSAY

This assay applies the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific to IL-13 Rα2 has been pre-coated onto a microplate. Standards and samples are then added to the appropriate plate wells with a biotin - conjugated antibody preparation specific for IL-13 Rα2 and incubated. IL-13 Rα2 if present, will bind and become immobilized by the antibody pre-coated on the wells and then be "sandwiched" by biotin conjugate. Avidin is a tetramer containing four identical subunits that each has a high affinity-binding site for biotin. After washing away any unbound substances, avidin-Horseradish Peroxidase (HRP) will be added to each well and incubated. Following a wash to remove any unbound antibody-enzyme reagent, a substrate solution is added to the wells and only those wells that contain IL-13 Rα2, biotin

conjugated antibody and enzyme-conjugated Avidin will exhibit a change in colour. The colour development is stopped and the intensity of the colour is measured.

In order to measure the concentration of IL-13 Rα2 in the samples, this package of reagent includes two calibration diluents (Calibrator Diluent I for serum/plasma testing and Calibrator Diluent II for cell culture supernatant testing.) According to the testing system, the provided standard is diluted (2-fold) with the appropriate Calibrator Diluent and assayed at the same time as the samples. This allows the operator to produce a standard curve of Optical Density (O.D) versus IL-13 Rα2 concentration. The concentration of IL-13 Rα2 in the samples is then determined by comparing the O.D. of the samples to the standard curve.

LIMITATIONS OF THE PROCEDURE

- FOR LABORATORY RESEARCH USE ONLY, NOT FOR USE IN DIAGNOSTIC PROCEDURES.
- The kit should not be used beyond the expiration date on the reagent label.
- It is important that the Calibration 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.
- As manufacturers we take great care to ensure that our products are suitable for use with all validated sample types, as designated in the product insert. However, it is possible that in some cases, high levels of interfering factors may cause unusual results.
- Any variation in standard diluent, operator, pipetting technique, washing technique, incubation time or temperature, and reagent age can cause variation in binding.
- Soluble receptors or other binding proteins present in biological samples do not necessarily interfere with the measurement of ligands in samples. However, until the factors have been tested, the possibility of interference cannot be excluded.

REAGENTS PROVIDED

All reagents provided are stored at 2-8°C. Refer to the expiration date on the label.

	96 tests

1. **IL-13 Rα2 Microtiter Plate** (Part EL39-1) ***96 wells***
   Pre-coated with anti-human IL-13 Rα2 monoclonal antibody.

2. **Biotin Conjugate** (Part EL39-2) ***6 mL***
   Anti-IL-13 Rα2 polyclonal antibody conjugated to Biotin

3. **Avidin Conjugate** (Part EL39-3) ***12 mL***
   Avidin conjugate to horseradish peroxidase

4. **IL-13 Rα2 STANDARD** (Part EL39-4) ***2 vials***
   Recombinant human IL-13 Rα2 (12.8ng/vial) in a buffered protein base with Preservative, lyophilized.

5. **CALIBRATOR DILUENT I** (Part EL39-5) ***25 mL***
   Animal serum with buffer and preservative. *For serum/plasma testing.*

6. **CALIBRATOR DILUENT II** (Part EL39-6) ***25 mL***
   Cell culture medium with calf serum and preservative. *For cell culture supernatant testing.*

7. **WASH BUFFER (20X)** (Part 30005) ***60 mL***
   20-fold concentrated solution of buffered surfactant.

8. **SUBSTRATE A** (Part EL39-7) ***10 mL***
   Buffered solution with H_2O_2

9. **SUBSTRATE B** (Part 30007) ***10 mL***
   Buffered solution with TMB.

10. **STOP SOLUTION** (Part 30008) ***14 mL***
    2N Sulphuric Acid (H_2SO_4). Caution: Caustic Material!

MATERIALS REQUIRED BUT NOT SUPPLIED

1. Single or multi-channel precision pipettes with disposable tips: 10-100µL and 50-200µL for running the assay.
2. Pipettes: 1 mL, 5 mL 10 mL, and 25 mL for reagent preparation.
3. Multi-channel pipette reservoir or equivalent reagent container.
4. Test tubes and racks.
5. Polypropylene tubes or containers (25 mL).
6. Erlenmeyer flasks: 100 mL, 400 mL, 1 L and 2 L.
7. Microtiter plate reader (450 nm ± 2nm).
8. Automatic microtiter plate washer or squirt bottle.
9. Sodium hypochlorite solution, 5.25% (household liquid bleach).
10. Deionized or distilled water.
11. Plastic plate cover.
12. Disposable gloves.
13. Absorbent paper.

PRECAUTIONS

1. Do not substitute reagents from one kit lot to another. Standard, conjugate and microtiter plates are matched for optimal performance. Use only the reagents supplied by manufacturer.
2. Allow kit reagents and materials to reach room temperature (20-25°C) before use. Do not use water baths to thaw samples or reagents.
3. Do not use kit components beyond their expiration date.
4. Use only deionized or distilled water to dilute reagents.
5. Do not remove microtiter plate from the storage bag until needed. Unused strips should be stored at 2-8°C in their pouch with the desiccant provided.
6. Use fresh disposable pipette tips for each transfer to avoid contamination.
7. Do not mix acid and sodium hypochlorite solutions.
8. Human serum and plasma should be handled as potentially hazardous and capable of transmitting disease. Disposable gloves must be worn during the assay procedure since no known test method can offer complete assurance that products derived from human blood will not transmit infectious agents. Therefore, all blood derivatives should be considered potentially infectious and good laboratory practices should be followed.
9. All samples should be disposed of in a manner that will inactivate human viruses.
   Solid Wastes: Autoclave for 60 minutes at 121°C.
   Liquid Wastes: Add sodium hypochlorite to a final concentration of 1.0%. The waste should be allowed to stand for a minimum of 30 minutes to inactivate viruses before disposal.
10. Substrate Solution is easily contaminated. If bluish prior to use, *do not use*.
11. Substrate B contains 20% acetone: Keep this reagent away from sources of heat and flame.
12. If Wash Buffer (20X) is stored at a lower temperature (2-5°C), crystals may form which must be dissolved by warming to 37°C prior to use.

SAMPLE PREPARATION

COLLECTION, HANDLING AND STORAGE

a) **Cell Culture Supernatant:** Centrifuge to remove any visible particulate material.
b) **Serum**: Blood should be drawn using standard venipuncture techniques and serum separated from the blood cells as soon as possible. Samples should be allowed to clot for one hour at room temperature, centrifuged for 10 minutes (4°C), and serum extracted.
a) **Plasma:** Blood should be drawn using standard venipuncture techniques and plasma collected using sodium citrate, EDTA, or heparin as an anticoagulant. To ensure optimal recovery and minimal platelet contamination, separation of plasma must be done on ice in less than 30 minutes after collection. Centrifuge for 10 minutes (4°C) to remove any particulate. *This IL-13 Rα2 ELISA kit is not affected by haemolysis of specimens. No adverse effects have been noted in the presence of anti-coagulants, sodium citrate, EDTA, or heparin*.

- Avoid grossly hemolytic, lipidic or turbid samples.
- Serum, plasma, and cell culture supernatant to be used within 24-48 hours may be stored at 2-8°C, otherwise samples must stored at -20°C to avoid loss of bioactivity and contamination. Avoid freeze-thaw cycles.
- When performing the assay, slowly bring samples to room temperature.
- It is recommended that all samples be assayed in duplicate.
- DO NOT USE HEAT-TREATED SPECIMENS.

PREPARATION OF REAGENTS

Remove all kit reagents from refrigerator and allow them to reach room temperature (20-25°C). Prepare the following reagents as indicated below. Mix thoroughly by gently swirling before pipetting. Avoid foaming.

1. **Wash Buffer (1X):** Add 60 mL of Wash Buffer (20X) and dilute to a final volume of 1200 mL with distilled or deionized water. Mix thoroughly. If a smaller volume of Wash Buffer (1X) is desired, add 1 volume of Wash Buffer (20X) to 19 volumes of distilled or deionized water. Wash Buffer (1X) is stable for 1 month at 2-8°C. Mix well before use.

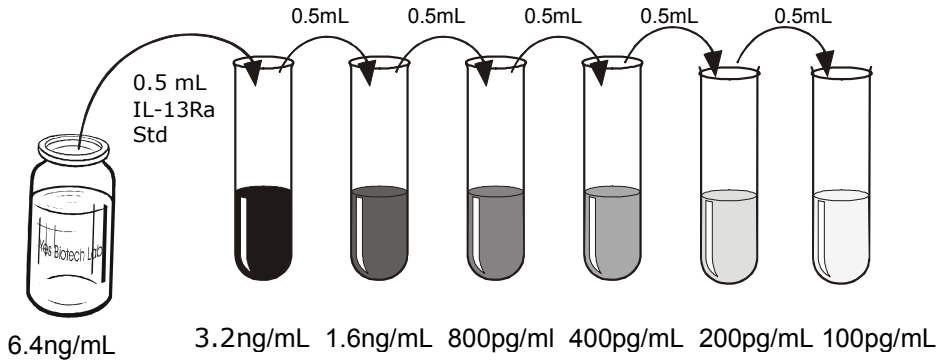
2. **Substrate Solution:** Substrate A and Substrate B should be mixed together in equal volumes up to 15 minutes before use. Refer to the table provided for correct amounts of Substrate Solution to prepare.

Strips Used	Substrate A (mL)	Substrate B (mL)	Substrate Solution (mL)
2 strips (16 wells)	2.0	2.0	4.0
4 strips (32 wells)	3.0	3.0	6.0
6 strips (48 wells)	4.0	4.0	8.0
8 strips (64 wells)	5.0	5.0	10.0
10 strips (80 wells)	6.0	6.0	12.0
12 strips (96 wells)	7.0	7.0	14.0

3. **IL-13 Rα2 Standard:**

a) Two vials of Standard are provided in this package to allow both serum/plasma and cell culture supernatant testing. Reconstitute IL-13 Rα2 Standard with 2.0 mL of Calibrator Diluent I or Calibrator Diluent II. This reconstitution produces a stock solution of 8 ng/mL. Allow solution to sit for at least 15 minutes with gentle agitation prior to making dilutions. Use within one hour of reconstituting. The IL-13 Rα2 standard stock solution can be stored frozen (-20°C) for up to 10 days. Avoid freeze-thaw cycles: aliquot if repeated use is expected.

b) Use the above stock solution to produce a serial 2-fold dilution series, as described below, within the range of this assay (100pg/mL to 6.4ng/mL) as illustrated. Add 0.5 mL of the appropriate Calibrator Diluent to each test tube. Between each test tube transfer, be sure to mix contents thoroughly. The undiluted IL-13 Rα2 stock solution will serve as the high standard (6.4ng/mL) and the Calibrator Diluent will serve as the zero standard (0ng/mL).



ASSAY PROCEDURE:

1. Prepare Wash Buffer (1X) and IL-13Ra2 Standards before starting assay procedure (see Preparation of Reagents). *It is recommended that the table and diagram provided be used as a reference for adding Standards or Samples to the Microtiter Plate.*

Wells	Contents	**Wells**	Contents
1A, 1B	Standard 1 - **0 pg/mL** (S1)	**2C, 2D**	Standard 6 - **1.6 ng/mL** (S6)
1C, 1D	Standard 2 – **100 pg/mL** (S2)	**2E, 2F**	Standard 7 - **3.2 ng/mL** (S7)
1E, 1F	Standard 3 – **200 pg/mL** (S3)	**2G, 2H**	Standard 8 - **6.4 ng/mL** (S8)
1G, 1H	Standard 4 – **400 pg/mL** (S4)	**3A-12H**	**IL-13 Rα2** samples
2A, 2B	Standard 5 – **800 pg/mL** (S5)		

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
A	S1	S5	1	5	9	13	17	21	25	29	32	36
B	S1	S5	1	5	9	13	17	21	25	29	32	37
C	S2	S6	2	6	10	14	18	22	26	30	33	37
D	S2	S6	2	6	10	14	18	22	26	30	33	38
E	S3	S7	3	7	11	15	19	23	27	31	34	39
F	S3	S7	3	7	11	15	19	23	27	31	34	39
G	S4	S8	4	8	12	16	20	24	28	32	35	40
H	S4	S8	4	8	12	16	20	24	28	32	35	40

2. Add 100µL of Standard or Sample to the appropriate well of the antibody pre-coated Microtiter Plate and incubate 1 hour at 37°C.

3. Without discarding the standards and samples, add 50µL IL-13 Rα2 Biotin conjugate to each wells. Mix well. Cover and incubate for 1 hour at 37°C.

4. Wash the Microtiter Plate using one of the specified methods indicated below:

   Manual Washing: Remove incubation mixture by aspirating contents of the plate into a sink or proper waste container. Using a squirt bottle, fill each well completely with Wash Buffer (1X) then aspirate contents of the plate into a sink or proper waste container. Repeat this procedure four more times for a **total of FIVE washes**. After final wash, invert plate, and blot dry by hitting plate onto absorbent paper or paper towels until no moisture appears. *Note*: Hold the sides of the plate frame firmly when washing the plate to assure that all strips remain securely in frame.
   Automated Washing: Aspirate all wells, then wash plates **FIVE times** using Wash Buffer (1X). Always adjust your washer to aspirate as much liquid as possible and set fill volume at 350 µL/well/wash (range: 350-400 µL). After final wash, invert plate, and blot dry by hitting plate onto absorbent paper or paper towels until no moisture appears. *It is recommended that the washer be set for a soaking time of 10 seconds or shaking time of 5 seconds between washes.*

5. Dispense 100µl of Avidin Conjugate to each well Mix well. Cover and incubate for 1 hour at 37°C.

6. Prepare Substrate Solution no more than 15 minutes before end of second incubation (see Preparation of Reagents).

7. Repeat wash procedure as described in Step 4.
8. Add 100µL Substrate Solution to each well. Cover and incubate for 15-20 minutes at 37°C.
9. Add 100µL Stop Solution to each well. Mix well.
10. Read the Optical Density (O.D.) at 450 nm using a microtiter plate reader set within 30 minutes.

CALCULATION OF RESULTS

The standard curve is used to determine the amount of IL-13 Rα2 in an unknown sample. The standard curve is generated by plotting the average O.D. (450 nm) obtained for each of the standard concentrations on the vertical (Y) axis versus the corresponding IL-13 Rα2 concentration (ng/mL) on the horizontal (X) axis.

1. First, calculate the mean O.D value for each standard and sample. All O.D. values are subtracted by the value of the zero-standard (0 ng/mL) or (S1) before result interpretation. Construct the standard curve using graph paper or statistical software.

2. To determine the amount of IL-13 Rα2 in each sample, first locate the O.D. value on the Y-axis and extend a horizontal line to the standard curve. At the point of intersection, draw a vertical line to the X-axis and read the corresponding IL-13 Rα2 concentration. If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

3. If samples generate values higher than the highest standard, dilute the samples and repeat the assay.

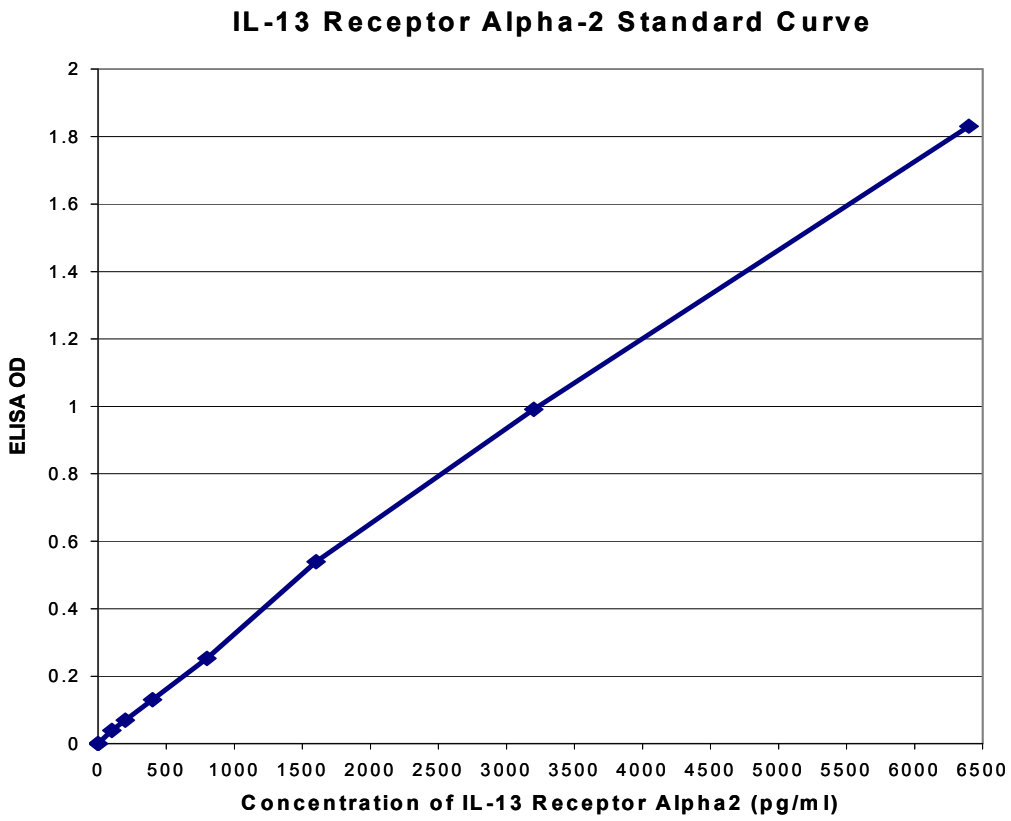
TYPICAL DATA

Results of a typical standard run of an IL-13 Rα2 ELISA are shown below. Any variation in standard diluent, operator, pipetting and washing technique, incubation time or temperature, and kit age can cause variation in result. The following examples are for the purpose of *illustration only*, and should not be used to calculate unknowns. Each user should obtain its own result.

EXAMPLE

The following data was obtained for a standard curve using Calibrator Diluent II.

Standard (pg/mL)	Mean O.D. (450 nm)	%CV	Zero Standard Subtracted (Std.) - (S1)
0	0.0945	6.73	0.000
100	0.1335	0.53	0.039
200	0.1640	0.00	0.069
400	0.2245	2.20	0.130
800	0.3470	5.60	0.253
1600	0.6290	4.92	0.535
3200	1.0850	3.13	0.991
6400	1.9260	2.73	1.831



SENSITIVITY

The sensitivity of human IL-13 Ra as observed by the standard curve generated for both Calibratory Diluent 1 and Calibrator Diluent II is about 25pg/ml. It is defined as the detected quantity 2SD from the mean OD of 16 replicates of the zero standard.

SPECIFICITY

This sandwich ELISA recognises both natural and recombinant human IL-13 Ra2. This kit has been tested and exhibited no significant cross-reactivity with following cytokines and growth factors: IL-1α, IL-1β, IL-2, IL-4, IL-5, Il-6, IL-7, IL-8, IL-10, IL-11, Il-12, Il-17A, FGF basic, GM-CSF, IFN-γ, M-CSF, MCP-1(MCAF), MCP3, EGF, TNF- α, TNF-β

REFERENCES

1. Biswajit Roy et al. IL-13 signal transduction in human monocytes: phosphorylation of receptor components, association with Jaks, and phosphorylation/activation of Stats. Journal of Leukocyte Biology 2002 Vol. 72: 580-589

2. Brigitte Granel et al. IL-13Ra2 gene polymorphisims are associated with systemic sclerosis. The Journal of Rheumatology. 2006 Vol. 33 102015-2019.

3. Edwin W Lai, Bharat H Joshi, Lucia Martiniova et al. Overexpression of interleukin-13 receptor 2 in neuroendocrine malignant pheochromocytoma: A novel target for Receptor directed anti-cancer therapy. The Journal of Clinical Endocrinology & Metabolism. 2009 Vol. 94, No. 8 2952-2957.

4. Makoto Saka et al. Vaccine therapy with dendritic cells transfected with IL13 Ra2 mRNA for glima in mice. The Journal of Neurosurgery. 2010 Vol. 113(2):270-9.

5. Mitomu Kioi et al. Interleukin-13 receptor alpha2 chain: a potential biomarker and molecular target for ovarian cancer therapy. Cancer 2006 Vol. 107(6): 1407–18

CITATIONS

1. Young Trieu et al. Soluble Interleukin-13Rα2 Decoy Receptor Inhibits Hodgkin's Lymphoma Growth in Vitro and in Vivo. Cancer Res May 1, 2004 64; 3271.