DRG® Interleukin-13 (rat) ELISA (EIA-3391)

Revised 28 June 2005 **For Veterinary Use Only**

INTRODUCTION

IL-13 is a T cell-derived cytokine that regulates the function of monocytes, macrophages, and B cells (1). IL-13 is secreted as a monomeric protein that shares 25 to 30 percent homology with interleukin-4. The human IL-13 protein has 132 amino acids and four potential N-linked glycosylation sites. The rat IL-13 protein is 131 amino acids in length and contains a 21 amino acid signal sequence (2). IL-13 is likely to have an anti-parallel four á-helical bundle similar to IL-4. In humans, the IL-13 gene is located on chromosome 5q31. This chromosomal region also contains the IL-3, IL-4, IL-5, and GM-CSF genes. IL-13 has four exon sequences that are contained within approximately 3 kb. Both IL-13 and IL-4 are produced by activated Th2 cells and target similar cells with the exception that only IL-4 affects T lymphocytes (3). IL-13 inhibits the production of the inflammatory cytokines IL-1â, IL-6, IL-8, and TNF-á by LPS-stimulated monocytes (4,5). In B cells, IL-13 induces differentiation and proliferation, promotes CD23 expression, and the production of certain immunoglobulin isotypes such as IgE. In monocytes it induces morphological changes with an upregulation of CD23, several members of the integrin superfamily, and MHC class II proteins. IL-13 stimulates macrophage-like cell production from bone marrow precursors and is a potent stimulator of Eotaxin.

INTENDED USE

The DRG Rat Interleukin-13 (Rat IL-13) ELISA is to be used for the *in vitro* quantitative determination of rat IL-13 in serum, plasma, buffered solution, or cell culture medium. The assay will recognize both natural and recombinant rat IL-13.

This kit has been configured for research use only and is not to be used in diagnostic procedures.

Read entire protocol before use.

PRINCIPLE OF THE METHOD

The DRG. Rat IL-13 kit is a solid phase sandwich Enzyme Linked-Immuno-Sorbent Assay (ELISA). A monoclonal antibody specific for rat IL-13 has been coated onto the wells of the microtiter strips provided. Samples, including standards of known IL-13 content, control specimens, and unknowns, are pipetted into these wells followed by a biotinylated second monoclonal antibody.

During the first incubation, the IL-13 antigen binds simultaneously to the immobilized (capture) antibody on one site, and to the solution phase biotinylated antibody on a second site.

After removal of excess second antibody, Streptavidin-Peroxidase (enzyme) is added. This binds to the biotinylated antibody to complete the four-member sandwich. After a second incubation and washing to remove all the unbound enzyme, a substrate solution is added, which is acted upon by the bound enzyme to produce color. The intensity of this colored product is directly proportional to the concentration of IL-13 present in the original specimen.

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REAGENTS PROVIDED

Note: *Store all reagents at 2 -8°C.*

Reagents	***96 Test Kit***	***192 Test Kit***
Rat IL-13 Standard, recombinant rat IL-13. Refer to vial label for quantity and reconstitution volume.	2 vials	4 vials
Standard Diluent Buffer, 25 mL per bottle. Contains 3.3 mM thymol and 8 mM sodium azide.	1 bottle	2 bottles
Rat IL-13 High and Low Control, recombinant rat IL-13, lyophilized. Refer to vial label for quantity and reconstitution volume. Once reconstituted, aliquot and store at -20°C or below. Avoid repeated freeze-thaw cycles.	2 vials	2 vials
Rat IL-13 Antibody-Coated Wells, 96 wells per plate.	1 plate	2 plates
Rat IL-13 Biotin Conjugate, (Biotin-labeled anti-rat IL-13), 15 mL per bottle. Contains 15 mM sodium azide.	1 bottle	2 bottles
Streptavidin-Peroxidase (HRP), (100x concentrate). 0.125 mL per vial. Contains 3.3 mM thymol.	1 vial	2 vials
Streptavidin-Peroxidase (HRP) Diluent, 25 mL per bottle. Contains 3.3 mM thymol.	1 bottle 1 bottle	
Wash Buffer Concentrate (25x). 100 mL per bottle.	1 bottle	1 bottle
Stabilized Chromogen, Tetramethylbenzidine (TMB), 25 mL per bottle.	1 bottle	1 bottle
Stop Solution, 25 mL per bottle.	1 bottle	1 bottle
Plate Covers, adhesive strips.	3	4

Disposal Note: This kit contains materials with small quantities of sodium azide. Sodium azide reacts with lead and copper plumbing to form explosive metal azides. Upon disposal, flush drains with a large volume of water to prevent azide accumulation. Avoid ingestion and contact with eyes, skin and mucous membranes. In case of contact, rinse affected area with plenty of water. Observe all federal, state and local regulations for disposal.

SUPPLIES - NOT PROVIDED

1. Microtiter plate reader capable of measurement at or near 450 nm.
2. Calibrated adjustable precision pipettes, preferably with disposable plastic tips. (A manifold multi-channel pipette is desirable for large assays.)
3. Deionized or distilled H2O.
4. Plate washer: automated or manual (squirt bottle, manifold dispenser, etc.).
5. Graph paper: linear (Cartesian), log-log, or semilog, as desired.
6. Glass or plastic tubes for diluting and aliquoting standard.
7. Absorbent paper towels.
8. Calibrated beakers and graduated cylinders in various sizes.

PROCEDURAL NOTES/LAB QUALITY CONTROL

1. When not in use, kit components should be refrigerated. All reagents should be warmed to room temperature before use.

2. **Microtiter plates should be allowed to come to room temperature before opening the foil bags.** Once the desired 8 number of strips has been removed, immediately reseal the bag with the desiccant and store at 2 - 8°C to maintain plate integrity.
3. Samples should be collected in pyrogen/endotoxin-free tubes.
4. Samples should be frozen if not analyzed shortly after collection. Avoid multiple freeze-thaw cycles of frozen samples. Thaw completely and mix well prior to analysis.
5. When possible, avoid use of badly hemolyzed or lipemic sera. If large amounts of particulate matter are present, centrifuge or filter prior to analysis.
6. It is recommended that all standards, controls and samples be run in duplicate.
7. Samples that are >500 pg/mL should be further diluted with *Standard Diluent Buffer.*
8. When pipetting reagents, maintain a consistent order of addition from well-to-well. This ensures equal incubation times for all wells.
9. Cover or cap all reagents when not in use.
10. **Do not mix or interchange different reagent lots from various kit lots**.
11. Do not use reagents after the kit expiration date.
12. Read absorbances within 2 hours of assay completion.
13. The provided controls should be run with every assay. If control values fall outside pre-established ranges, the accuracy of the assay is suspect.
14. All residual wash liquid must be drained from the wells by efficient aspiration or by decantation followed by tapping the plate forcefully on absorbent paper. ***Never*** insert absorbent paper directly into the wells.
15. Because *Stabilized Chromogen* is light sensitive, avoid prolonged exposure to light. Also avoid contact between *Stabilized Chromogen* and metal, or color may develop.

SAFETY

All blood components and biological materials should be handled as potentially hazardous. Follow universal precautions as established by the Center for Disease Control and Prevention and by the Occupational Safety and Health Administration when handling and disposing infectious agents.

DIRECTIONS FOR WASHING

Incomplete washing will adversely affect the test outcome. All washing must be performed with *Wash Buffer* provided. Washing can be performed manually as follows: completely aspirate the liquid from all wells by gently lowering an aspiration tip (aspiration device) into the bottom of each well. Take care not to scratch the inside of the well. After aspiration, fill the wells with at least 0.4 mL of diluted wash solution. Let soak for 15 to 30 seconds, then aspirate the liquid. Repeat as directed under **ASSAY METHOD**. After the washing procedure, the plate is inverted and tapped dry on absorbent tissue. Alternatively, the wash solution may be put into a squirt bottle. If a squirt bottle is used, flood the plate with wash buffer, completely filling all wells. After the washing procedure, the plate is inverted and tapped dry on absorbent tissue. If using an automated washer, the operating instructions for washing equipment should be carefully followed. High pressure jets of some automated washers may adversely affect reproducibility.

REAGENT PREPARATION AND STORAGE

A. Reconstitution and Dilution of IL-13 Standard

Note: Either glass or plastic tubes may be used for standard dilutions.

1. Reconstitute the standard to 10,000 pg/mL with *Standard Diluent Buffer*. Refer to the standard vial label for instructions. Swirl gently and allow to sit for 10 minutes to ensure complete reconstitution.
2. Add 0.050 mL of the reconstituted standard to a tube containing 0.950 mL *Standard Diluent Buffer*. Label as 500 pg/mL IL-13. Mix.
3. Add 0.300 mL of *Standard Diluent Buffer* to each of 6 tubes labeled 250, 125, 62.5, 31.3, 15.6 and 7.8 pg/mL IL-13.

4. Make serial dilutions of the standard as described in the following dilution table. Mix thoroughly between steps.

B. Dilution of IL-13 Standard

Standard:	**Add:**	**Into:**
500 pg/mL	Prepare as described in Step 2	
250 pg/mL	0.300 mL of the 500 pg/mL std.	0.300 mL of the Diluent Buffer
125 pg/mL	0.300 mL of the 250 pg/mL std.	0.300 mL of the Diluent Buffer
62.5 pg/mL	0.300 mL of the 125 pg/mL std.	0.300 mL of the Diluent Buffer
31.3 pg/mL	0.300 mL of the 62.5 pg/mL std.	0.300 mL of the Diluent Buffer
15.6 pg/mL	0.300 mL of the 31.3 pg/mL std.	0.300 mL of the Diluent Buffer
7.8 pg/mL	0.300 mL of the 15.6 pg/mL std.	0.300 mL of the Diluent Buffer
0 pg/mL	0.300 mL of the Diluent Buffer	An empty tube

Discard all remaining reconstituted and diluted standards after completing assay. Return *Standard Diluent Buffer* to the refrigerator.

C. Storage and Final Dilution of Streptavidin-HRP
Please Note: The *Streptavidin-HRP* 100x concentrate is in 50% glycerol. This solution is viscous. To ensure accurate dilution, allow *Streptavidin-HRP* concentrate to reach room temperature. Gently mix. Pipette *Streptavidin-HRP* concentrate slowly. Remove excess concentrate solution from pipette tip by gently wiping with clean absorbent paper.

1. Dilute 10 µL of the 100x concentrated solution with 1 mL of *Streptavidin-HRP Diluent* for each 8-well strip used in the assay. Label as Streptavidin-HRP Working Solution.

For Example:

# of 8-Well Strips	Volume of Streptavidin-HRP Concentrate		Volume of Diluent
2	20 µL solution	2 mL	
4	40 µL solution		4 mL
6	60 µL solution	6 mL	
8	80 µL solution	8 mL	
10	100 µL solution		10 mL
12	120 µL solution		12 mL

2. Return the unused *Streptavidin-HRP* concentrate to the refrigerator.

D. Dilution of Wash Buffer
Allow the 25x concentrate to reach room temperature and mix to ensure that any precipitated salts have redissolved. Dilute 1 volume of the 25x wash buffer concentrate with 24 volumes of deionized water (e.g., 50 mL may be diluted up to 1.25 liters, 100 mL may be diluted up to 2.5 liters). Label as Working Wash Buffer.

Store both the concentrate and the Working Wash Buffer in the refrigerator. The diluted buffer should be used within 14 days.

ASSAY METHOD: PROCEDURE AND CALCULATIONS

Be sure to read the *Procedural Notes/Lab Quality Control* section before carrying out the assay.

Allow all reagents to reach room temperature before use. Gently mix all liquid reagents prior to use.

Note: A standard curve must be run with each assay.

1. Determine the number of 8-well strips needed for the assay. Insert these in the frame(s) for current use. (Re-bag extra strips and frame. Store these in the refrigerator for future use.)
2. Add 50 µL of *Standard Diluent Buffer* to zero wells. Well(s) reserved for chromogen blank should be left empty.
3. Add 50 µL of standards or controls to the appropriate microtiter wells. For buffered solution or cell culture samples, add 50 µL sample to each well. For serum or plasma samples, add 25 µL of *Standard Diluent Buffer* to each well followed by 25 µL of sample. (See **REAGENT PREPARATION AND STORAGE**, Section B.)
4. Pipette 150 µL of biotinylated anti-rat IL-13 (*Biotin Conjugate*) solution into each well except the chromogen blank(s). Mix thoroughly on a plate shaker or by gently tapping on the side of the plate.
5. Cover plate with *plate cover* and incubate for **2 hours at room temperature**.
6. Thoroughly aspirate or decant solution from wells and discard the liquid. Gently wash wells 4 times. See **DIRECTIONS FOR WASHING**.
7. Add 100 µL Streptavidin-HRP Working Solution to each well except the chromogen blank(s). (Prepare the working dilution as described in **REAGENT PREPARATION AND STORAGE,** Section C.)
8. Cover plate with the *plate cover* and incubate for **30 minutes at room temperature**.
9. Thoroughly aspirate or decant solution from wells and discard the liquid. Gently wash wells 4 times. See **DIRECTIONS FOR WASHING**.
10. Add 100 µL of *Stabilized Chromogen* to each well. The liquid in the wells will begin to turn blue.
11. Incubate for **30 minutes at room temperature and in the dark**. ***Please Note*: Do not cover the plate with aluminum foil or metalized mylar**. The microtiter plate reader used often determines the incubation time for chromogen substrate. Many plate readers have the capacity to record a maximum optical density (O.D.) of 2.0. The O.D. values should be monitored and the substrate reaction stopped before the O.D. of the positive wells exceed the limits of the instrument. The O.D. values at 450 nm can only be read after the *Stop Solution* has been added to each well. If using a reader that records only to 2.0 O.D., stopping the assay after 20 to 25 minutes is suggested.
12. Add 100 µL of *Stop Solution* to each well. Tap side of plate gently to mix. The solution in the wells should change from blue to yellow.
13. Read the absorbance of each well at 450 nm having blanked the plate reader against a chromogen blank composed of 100 µL eachof *Stabilized Chromogen* and *Stop Solution*. Read the plate within 2 hours after adding the *Stop Solution*.
14. Plot on graph paper the absorbance of the standards against the standard concentration. (Optimally, the background absorbance may be subtracted from *all* data points, including standards, unknowns and controls, prior to plotting.) Draw the best smooth curve through these points to construct the standard curve. If using curve fitting software, the four parameter algorithm usually provides the best curve fit.
15. Read the IL-13 concentrations for unknown samples and controls from the standard curve plotted in Step 14. **Multiply value(s) obtained for serum or plasma sample(s) by 2 to correct for the 1:2 dilution in Step 3**. Samples producing signals greater than that of the highest standard (500 pg/mL) should be diluted in *Standard Diluent Buffer* and re-analyzed, multiplying the concentration found by the appropriate dilution factor.

TYPICAL DATA

The following data were obtained for the various standards over the range of 0 to 500 pg/mL IL-13.

Standard rat IL-13 (pg/mL)	Optical Density (450 nm)
0	0.093
	0.093
7.8	0.153
	0.155
15.6	0.203
	0.200
31.3	0.288
	0.279
62.5	0.445
	0.447
125	0.806
	0.818
250	1.415
	1.425
500	2.606
	2.550

LIMITATIONS OF THE PROCEDURE

Do not extrapolate the standard curve beyond the 500 pg/mL standard point; the dose-response is non-linear in this region and accuracy is difficult to obtain. Dilute samples >500 pg/mL with *Standard Diluent Buffer*. Re-analyze these and multiply results by the appropriate dilution factor.

The influence of various drugs, aberrant sera (hemolyzed, hyperlipidemic, jaundiced, etc.) and the use of biological fluids in place of serum samples have not been thoroughly investigated. The rate of degradation of native IL-13 in various matrices has not been investigated. The immunoassay literature contains frequent references to aberrant signals seen with some sera, attributed to heterophilic antibodies, binding molecules and soluble receptors. Though such samples have not been seen to date, the possibility of this occurrence cannot be excluded.

This kit is for research use only.
Not for human therapeutic or diagnostic use.

PERFORMANCE CHARACTERISTICS

SENSITIVITY

The minimum detectable dose of IL-13 is 1.5 pg/mL. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 44 times.

PRECISION

1. Intra-Assay Precision

Samples of known IL-13 concentration were assayed in replicates of 24 to determine precision within an assay.

	Sample 1	Sample 2	Sample 3
Mean (pg/mL) 23.6	87.8	353	
SD	1.3	3.7	9.7
%CV	5.5	4.2	2.7

SD = Standard Deviation
CV = Coefficient of Variation

2. Inter-Assay Precision
Samples were assayed 12 times in multiple assays to determine precision between assays.

	Sample 1	Sample 2	Sample 3
Mean (pg/mL)	14	80	308
SD	1.2	3.0	23.7
%CV	8.3	3.8	7.7

SD = Standard Deviation
CV = Coefficient of Variation

LINEARITY OF DILUTION
Pools of rat sera, EDTA plasma, heparin plasma (pool of 3 samples) and RPMI containing 10% fetal calf serum (FCS) or 1% FCS were spiked with 360 pg/mL of IL-13 and serially diluted in *Standard Diluent Buffer* over the range of the assay. Linear regression analysis of samples versus the expected concentration yielded correlation coefficients greater than 0.99.

RECOVERY
The recovery of IL-13 added to tissue culture medium containing 10% fetal calf serum (FCS) or 1% FCS averaged 104% and 99.6%, respectively. Recoveries of IL-13 spiked into 12 sera and 15 EDTA plasma averaged 100% (CV 4.0%) and 98% (CV 4.1%), respectively. The recovery of IL-13 added to three heparinized plasma was 103.3%.

SPECIFICITY
Buffered solutions of a panel of substances at 150 ng/mL were assayed with the DRG. Rat IL-13 kit. The following substances were tested and found to have no cross-reactivity: rat CINC-2β, IFN-γ, IL-1β, IL-2, IL-4, IL-6, IL-10, MIP-2, TNF-α; mouse IFN-γ, IL-1β, IL-2, IL-3, IL-4, IL-6, IL-10, TNF-α; human IL-10, IL-13, IL-15.

HIGH DOSE HOOK EFFECT
A sample spiked with 1200 ng/mL of IL-13 gave a response higher than that obtained for the last standard point.

EXPECTED VALUES
EDTA plasma (n=20) and sera (n=21) from normal, outbred, female rats were evaluated in this assay. The average values for 19 EDTA plasma and 20 sera were, respectively, 13.2 ± 4.7 pg/mL and 17.1 ± 8.5 pg/mL.
One additional serum and one additional EDTA plasma showed elevated values of 45 and 38 pg/mL, respectively. The lowest values for both types of samples were extrapolated to 4.0 pg/mL. Values of 5.7, 17 and 18 pg/mL were observed for the three heparin plasma assayed.
Cell culture supernatants were evaluated in this assay. Rat spleen cells were cultured in RPMI supplemented with 5% FCS for 4, 24, 48 or 72 hours either without stimulation, or with a blend of LPS (25 µg/mL) and PHA (5 µg/mL), or with a blend of ionomycin (100 ng/mL) and PMA (100 ng/mL). Results are shown below.

Levels of IL-13 from cultured Rat splenocytes.

	Rat IL-13 (pg/mL)			
Stimulation Condition	***4 hrs.***	***24 hrs.***	***48 hrs.***	***72 hrs.***
Unstimulated	<1.5	4.7	9.8	9.8
LPS + PHA	3.6	321	847	817
PMA + ionomycin	19.7	1080	1940	1500

REFERENCES

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