DRG® Interleukin 10 (mouse) (EIA-3298)

Revised 7 May 2007 **For Veterinary Use Only**

INTRODUCTION

Interleukin 10 is a pleiotropic cytokine acting on a variety of immune cells. IL-10 was first characterized as a cytokine synthesis inhibitory factor (CSIF) able to inhibit cytokine synthesis by TH1 clones activated in the presence of antigen presenting cells. However, in the absence of monocytes, IL-10 directly inhibits the growth of T cells triggered by immobilized anti-CD3 monoclonal antibody. This proliferation inhibition was found to be a result of specific inhibition of IL-2 production by the responding T-cells. *In vitro*, IL-10 is a very powerful inhibitor of monokines (including TNF-a , IL-1, IL-6, and IL-8) produced by LPS-activated monocytes and macrophages. The addition of IL-10 to B lymphocytes results in limited cell proliferation but most importantly, in very high immunoglobulin production, a result of the transformation of B cells into plasma cells. Finally, natural killer (NK) cells appear to be another target for the anti-inflammatory properties of IL-10. Data have shown that IL-10 can inhibit antigen-induced
IFN-y production by NK cells by inhibiting not only production but also the stimulatory effects of IL-12 and TNF on IFN-. production. Circulating levels of IL-10 have been found in serum of patients suffering from Non-Hodgkin's lymphoma, multiple myeloma, cerebral malaria or septic shock. (1-6) There is 72% protein homology between human and mouse mature IL-10. Mouse IL-10 is a 35 kDa non-disulfide linked homodimer, that is acid labile and rapidly loses activity below pH 5.5 (7). Citations for the mIL-10 kit are found in references 8-14.

INTENDED USE

The DRG Mouse Interleukin-10 (mIL-10) ELISA is to be used for the *in vitro* quantitative determination of mIL-10 in mouse serum, buffered solution, or cell culture medium. The assay will recognize both natural and recombinant mIL-10.
In the United States, this kit is intended for Research Use Only.

PRINCIPLE OF THE METHOD

The DRG mIL-10 kit is a solid phase sandwich Enzyme Linked-Immuno-Sorbent Assay (ELISA). A monoclonal antibody specific for mIL-10 has been coated onto the wells of the microtiter strips provided. Samples, including standards of known mIL-10 content, control specimens, and unknowns, are pipetted into these wells. During the first incubation, the mIL-10 antigen binds to the immobilized (capture) antibody on one site. After washing, a biotinylated monoclonal antibody specific for mIL-10 is added. During the second incubation, this antibody binds to the immobilized mIL-10 captured during the first incubation. 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 third 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 mIL-10 present in the original specimen.

REAGENTS PROVIDED

Note: *Store all reagents at 2 - 8°C.*

Reagent	***96Test Kit***	***192 Test Kit***
*mIL-10 Standar*d, recombinant mIL-10. Refer to vial label for quantity and reconstitution volume.	2 vials	4 vials
*Standard Diluent Buffe*r Contains 15 mM sodium azide; 25 mL per bottle.	1 bottle	2 bottles
*mIL-10 High and Low Contro*l, recombinant IL-10 in tissue culture matrix, lyophilized. Refer to vial label for quantity and reconstitution volume. Once reconstituted, aliquot and store at –20ºC or below. Avoid repeated freeze-thaw cycles.	2 vials	2 vials
*mIL-10 Antibody-Coated Well*s, 96 wells per plate.	1 plate	2 plates

mIL-10 Biotin Conjugate (Biotin-labeled anti-IL-10). Contains 15 mM sodium azide; 11 mL per bottle.	1 bottle	2 bottles
Streptavidin-Peroxidase (HRP), (100x) concentrate. Contains 3.3 mM thymol; 0.125 mL per vial.	1 vial	2 vials
Streptavidin-Peroxidase (HRP) Diluent. Contains 3.3 mM thymol; 25 mL per bottle	1 bottle	1 bottle
Wash Buffer Concentrate (25x); 100 mL per bottle.	1 bottle	1 bottle
*Stabilized Chromoge*n, Tetramethylbenzidine (TMB); 25 mL per bottle.	1 bottle	1 bottle
*Stop Solutio*n; 25 mL per bottle.	1 bottle	1 bottle
*Plate Cover*s, adhesive strips.	3	6

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.
9. 37°C incubator.

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
3. number of strips has been removed, immediately reseal the bag and store at 2 - 8°C to maintain plate integrity.
4. Samples should be collected in pyrogen/endotoxin-free tubes.
5. 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.
6. When possible, avoid use of badly hemolyzed or lipemic sera. If large amounts of particulate matter are present, centrifuge or filter prior to analysis.
7. It is recommended that all standards, controls and samples be run in duplicate.
8. Samples that are >2000 pg/mL should be diluted with *Standard Diluent Buffe*r.
9. When pipetting reagents, maintain a consistent order of addition from well-to-well. This ensures equal incubation times for all wells.
10. Cover or cap all reagents when not in use.
11. **Do not mix or interchange different reagent lots from various kit lot**s.
12. Do not use reagents after the kit expiration date.

13. Read absorbances within 2 hours of assay completion.
14. The provided controls should be run with every assay. If control values fall outside pre-established ranges, the accuracy of the assay is suspect.
15. 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.
16. 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 Centers 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 METHO**D. 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.

REAGENT PREPARATION AND STORAGE

A. Reconstitution and Dilution of mIL-10 Standard

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

1. Reconstitute standard to 10,000 pg/mL with *Standard Diluent Buffe*r. Refer to standard vial label for instructions. Swirl or mix gently and allow to sit for 10 minutes to ensure complete reconstitution. Use standard within 1 hour of reconstitution.
2. Add 0.200 mL of the reconstituted standard to a tube containing 0.800 mL *Standard Diluent Buffe*r. Label as 2000 pg/mL mIL-10. Mix.
3. Add 0.300 mL of *Standard Diluent Buffer* to each of 6 tubes labeled 1000, 500, 250, 125, 62.5, and 31.2 pg/mL mIL-10.
4. Make serial dilutions of the standard as described in the following dilution table. Mix thoroughly between steps.

B. Dilution of mIL-10 Standard

Standard:	Add:	Into:
2000 pg/mL	prepare as described in step 2	
1000 pg/mL	0.300 mL of the 2000 pg/mL std.	0.300 mL of the Diluent Buffer
500 pg/mL	0.300 mL of the 1000 pg/mL std.	0.300 mL of the Diluent Buffer
250 pg/mL	0.300 mL of the500 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
31.2 pg/mL	0.300 mL of the62.5 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 the *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 this 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

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 100 µL of the *Standard Diluent Buffer* to zero wells. Well(s) reserved for chromogen blank should be left empty.
3. Add 100 µL of standards, samples or controls to the appropriate microtiter wells. Tap gently on the side of plate to mix. (See **REAGENT PREPARATION AND STORAG**E, Section B.)
4. Cover plate with *plate cover* and incubate for **1 hour at 37º**C.
5. Thoroughly aspirate or decant solution from wells and discard the liquid. Wash wells 4 times. See **DIRECTIONS FOR WASHIN**G.
6. Pipette 100 µL of biotinylated anti-IL-10 *(Biotin Conjugat*e) solution into each well except the chromogen blank(s). Tap gently on the side of the plate to mix.
7. Cover plate with *plate cover* and incubate for **1 hour at room temperatur**e.
8. Thoroughly aspirate or decant solution from wells and discard the liquid. Wash wells 4 times. See **DIRECTIONS FOR WASHIN**G.
9. 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 STORAG**E, Section C.)
10. Cover plate with the *plate cover* and incubate for **30 minutes at room temperatur**e.
11. Thoroughly aspirate or decant solution from wells and discard the liquid. Wash wells 4 times. See **DIRECTIONS FOR WASHIN**G.
12. Add 100 µL of *Stabilized Chromogen* to each well. The liquid in the wells will begin to turn blue.
13. Incubate for **30 minutes at room temperature and in the dar**k.

    ***Please Note*: Do not cover the plate with aluminum foil or metalized mylar**. The incubation time for chromogen substrate is often determined by the microtiter plate reader used. 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.
14. 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.
15. Read the absorbance of each well at 450 nm having blanked the plate reader against a chromogen blank composed of 100 µL each of *Stabilized Chromogen* and *Stop Solution*. Read the plate within 2 hours after adding the *Stop Solution*.
16. 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 provides the best curve fit.
17. Read the mIL-10 concentrations for unknown samples and controls from the standard curve plotted in Step 16. (Samples producing signals greater than that of the highest standard (2000 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 2000 pg/mL mIL-10.

Standard mIL-10 (pg/mL)	Optical Density (450 nm)
0	0.083 0.087
31.2	0.115 0.107
62.5	0.170 0.161
125	0.267 0.251
250	0.421 0.366
500	0.752 0.681
1000	1.256 2.398

LIMITATIONS OF THE PROCEDURE

Do not extrapolate the standard curve beyond the 2000 pg/mL standard point; the dose-response is non-linear in this region and accuracy is difficult to obtain. Dilute samples >2000 pg/mL with *Standard Diluent Buffe*r; 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 mIL-10 in various matrices has not been investigated. The immunoassay literature contains frequent references to aberrant signals seen with some sera, attributed to heterophilic antibodies. Though such samples have not been seen to date, the possibility of this occurrence cannot be excluded.

PERFORMANCE CHARACTERISTICS

SENSITIVITY

The minimum detectable dose of mIL-10 is <13 pg/mL. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 30 times.

PRECISION

1. Intra-Assay Precision

Samples of known mIL-10 concentration were assayed in replicates of 14 to determine precision within an assay.

	Sample 1	**Sample 2**	**Sample 3**
Mean (pg/mL)	230.4	699.4	1466.7
SD	15.0	31.7	59.7
%CV	6.5	4.5	4.1

SD = Standard Deviation
CV = Coefficient of Variation

2. Inter-Assay Precision

Samples were assayed 42 times in multiple assays to determine precision between assays.

	Sample 1	**Sample 2**	**Sample 3**
Mean (pg/mL)	238.6	715.7	1477.3
SD	22.4	48.6	129.3
%CV	9.4	6.8	8.8

SD = Standard Deviation
CV = Coefficient of Variation

LINEARITY OF DILUTION

Mouse serum and tissue culture medium containing 10% fetal calf serum were spiked with mIL-10 and serially diluted in *Standard Diluent Buffer* over the range of the assay. Linear regression analysis of samples versus the expected concentration yielded a correlation coefficient of 0.99 in both cases.

Dilution	**Serum**			**Cell Culture**		
	Measured (pg/mL)	**Expected (pg/mL)**	**% Expected**	**Measured (pg/mL)**	**Expected (pg/mL)**	**% Expected**
Neat	1368	-	-	1566	-	-
1/2	703	684	103	712	783	91
1/4	354	342	104	313	392	80
1/8	188	171	110	167	196	85
1/16	94	86	109	83	98	85
1/32	52	43	121	42	49	86

RECOVERY

The recovery of mIL-10 added to mouse serum averaged 81%. The recovery of mIL-10 added to tissue culture medium containing 1% fetal calf serum averaged 104%, while the recovery of mIL-10 added to tissue culture medium containing 10% fetal calf serum averaged 102%.

SPECIFICITY

Buffered solutions of a panel of substances at 10,000 pg/mL were assayed with the DRG mIL-10 kit. The following substances were tested and found to have no cross-reactivity:
mouse IL-2, IL-3, IL-4, IL-6, IFN-y, TNF-a; rat IL-1, IL-2, IL-4, IL-6, IL-10, IFN-y, TNF-a ; human IL-2, IL-2R, IL-6, IL-8, IL-10, SCF; swine IL-10.

CULTURE STIMULATIONS

Mouse splenocytes were cultured under the following conditions and the culture supernatants were assayed for mIL-10 released.
1. Unstimulated 15 hr control: 11 pg/mL
2. Unstimulated 38 hr control: 0 pg/mL
3. Unstimulated 72 hr control: 46 pg/mL
4. Con-A (5 µg/mL) 15 hr: 159 pg/mL
5. Con-A (5 µg/mL) 38 hr: 360 pg/mL
6. Con-A (5 µg/mL) 72 hr: 852 pg/mL
7. LPS (10 µg/mL) 15 hr: 231 pg/mL
8. LPS (10 µg/mL) 38 hr: 787 pg/mL
9. LPS (10 µg/mL) 72 hr: 1450 pg/mL
10. PMA (50 ng/mL), Ionophore (250 ng/mL) 15 hr: 2 pg/mL
11. PMA (50 ng/mL), Ionophore (250 ng/mL) 38 hr: 37 pg/mL
12. PMA (50 ng/mL), Ionophore (250 ng/mL) 72 hr: 271 pg/mL

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