Quantikine®

Human IL-16 Immunoassay

Catalog Number D1600

For the quantitative determination of human interleukin 16 (IL-16) concentrations in cell culture supernates, 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.

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INTRODUCTION

Interleukin 16 (IL-16), formerly known as lymphocyte chemoattractant factor or LCF, is a proinflammatory cytokine that is chemotactic for $CD4^+$ T lymphocytes, monocytes and eosinophils (1, 2). In addition, IL-16 can upregulate IL-2 receptor and HLA-DR expression (3, 4), inhibit T cell receptor (TcR)/CD3-dependent activation (5, 6) and promote repression of HIV-1 transcription (7). IL-16 is a unique cytokine with no significant sequence homology to other well-characterized cytokines or chemokines.

IL-16 was originally identified as a homotetramer consisting of individual 14 kDa monomers of 130 amino acids (aa) each (8). It is synthesized as a precursor molecule (pro-IL-16) of approximately 68 kDa and 631 aa lacking a signal peptide (9, 10). The gene for human IL-16 maps to chromosome 15 and the sequence displays > 90% homology to those of various nonhuman primates (11, 12).

Recombinant pro-IL-16 polypeptides are specifically cleaved in $CD8^+$ cell lysates suggesting that the actual secreted form of IL-16 may be smaller than the originally published 130 aa form (9). In $CD8^+$ T cells, active caspase-3 cleaves pro-IL-16 producing a biologically active, secreted form of IL-16 (*i.e.* representing 121 C-terminal aa residues of pro-IL-16) (13). The mechanism of release or secretion of IL-16, however, is currently unknown but does not appear to correlate with apoptosis (14).

CD4 serves as a signal-transducing receptor for IL-16. Expression of CD4 is required for mediating IL-16 functions (3, 4, 15 - 17). Interaction between IL-16 and CD4 can specifically initiate an increase in intracytoplasmic calcium and inositol trisphosphate (3), activation of $p56^{lck}$ (15), and translocation of protein kinase C from the cytosol to the cell membrane (17). The region of CD4 that binds IL-16 has been identified within the D4 domain, overlapping the structure involved in CD4 dimer formation (18).

Sources of IL-16 include epithelial cells, mast cells, lymphocytes, macrophages, synovial fibroblasts, and eosinophils (14, 19 - 31). IL-16 mRNA is constitutively expressed in both $CD4^+$ and $CD8^+$ cells (28); however, transcription is induced in T lymphocytes upon exposure to antigen or mitogen (1). IL-16 may also be secreted by activated $CD8^+$ cells in response to histamine or serotonin. IL-16 expression has been linked to inflammation processes in asthma, rheumatoid arthritis, systemic lupus erythematosus, colitis, atopic dermatitis, and multiple sclerosis (20 - 24, 30, 32 - 38). For example, the expression of IL-16 directly correlates with the number of infiltrating $CD4^+$ T cells in asthmatic epithelium (22, 39).

The Quantikine Human IL-16 Immunoassay is a 4.5 hour solid phase ELISA designed to measure human IL-16 in cell culture supernates, serum, and plasma. It contains *E. coli*-expressed recombinant human IL-16 and antibodies raised against the recombinant factor. It has been shown to accurately quantitate recombinant human IL-16. Results obtained using natural IL-16 showed linear curves that were parallel to the standard curves obtained using the recombinant kit standards. These results indicate that the Quantikine Immunoassay kit can be used to determine relative mass values for natural human IL-16.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for IL-16 has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any IL-16 present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for IL-16 is added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, a substrate solution is added to the wells and color develops in proportion to the amount of IL-16 bound in the initial step. 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 with those from other lots or sources.
- If samples fall outside the dynamic range of the assay, dilute the samples with the appropriate Calibrator Diluent and repeat the assay.
- Any variation in standard diluent, operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.
- This assay is designed to eliminate interference by receptors or other proteins present in biological samples. Until all proteins have been tested in the Quantikine Immunoassay, the possibility of interference cannot be excluded.

MATERIALS PROVIDED

IL-16 Microplate (Part 890751) - 96 well polystyrene microplate (12 strips of 8 wells) coated with a mouse monoclonal antibody against IL-16.

IL-16 Conjugate (Part 890752) - 21 mL of polyclonal antibody against IL-16 conjugated to horseradish peroxidase with preservative.

IL-16 Standard (Part 890753) - 20 ng of recombinant human IL-16 in a buffered protein base with preservative; lyophilized.

Assay Diluent RD1W (Part 895117) - 11 mL of a buffered protein base with preservative.

Calibrator Diluent RD5R (Part 895190) - 21 mL of a buffered protein base with preservative. *For cell culture supernate samples.*

Calibrator Diluent RD6-27 (Part 895339) - 21 mL of animal serum with preservative. *For serum/plasma samples.*

Wash Buffer Concentrate (Part 895003) - 21 mL of a 25-fold concentrated solution of buffered surfactant with preservative.

Color Reagent A (Part 895000) - 12.5 mL of stabilized hydrogen peroxide.

Color Reagent B (Part 895001) - 12.5 mL of stabilized chromogen (tetramethylbenzidine).

Stop Solution (Part 895032) - 6 mL of 2 N sulfuric acid.

Plate Covers - 4 adhesive strips.

STORAGE

<table>
<tr><td>Unopened Kit</td><td colspan="2">Store at 2 - 8° C. Do not use past kit expiration date.</td></tr>
<tr><td rowspan="10">Opened/ Reconstituted Reagents</td><td>Diluted Wash Buffer</td><td rowspan="9">May be stored for up to 1 month at 2 - 8° C.*</td></tr>
<tr><td>Stop Solution</td></tr>
<tr><td>Assay Diluent RD1W</td></tr>
<tr><td>Calibrator Diluent RD5R</td></tr>
<tr><td>Calibrator Diluent RD6-27</td></tr>
<tr><td>Conjugate</td></tr>
<tr><td>Unmixed Color Reagent A</td></tr>
<tr><td>Unmixed Color Reagent B</td></tr>
<tr><td>Standard</td></tr>
<tr><td>Microplate Wells</td><td>Return unused wells to the foil pouch containing the desiccant pack, reseal along entire edge of zip-seal. May be stored for up to 1 month at 2 - 8° C.*</td></tr>
</table>

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

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.
- Test tubes for dilution.
- Human IL-16 Controls (optional; available from R&D Systems).

PRECAUTIONS

Calibrator Diluent RD6-27 contains sodium azide, which may react with lead and copper plumbing to form explosive metallic azides. Flush with large volumes of water during disposal.

The Stop Solution provided with this kit is an acid solution. Wear eye, hand, face, and clothing protection when using this material.

IL-16 is detectable in saliva and sweat. Take precautionary measures to prevent contamination of kit reagents while running the assay.

SAMPLE COLLECTION AND STORAGE

Cell Culture Supernates - Remove particulates by centrifugation and assay immediately or aliquot and store samples at ≤ -20° C. Avoid repeated freeze-thaw cycles.

Serum - Use a serum separator tube (SST) and allow samples to clot for 30 minutes 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 is not validated for use in this assay. Hemolyzed samples are not suitable for use in this assay.*

REAGENT PREPARATION

Bring all reagents to room temperature before use.

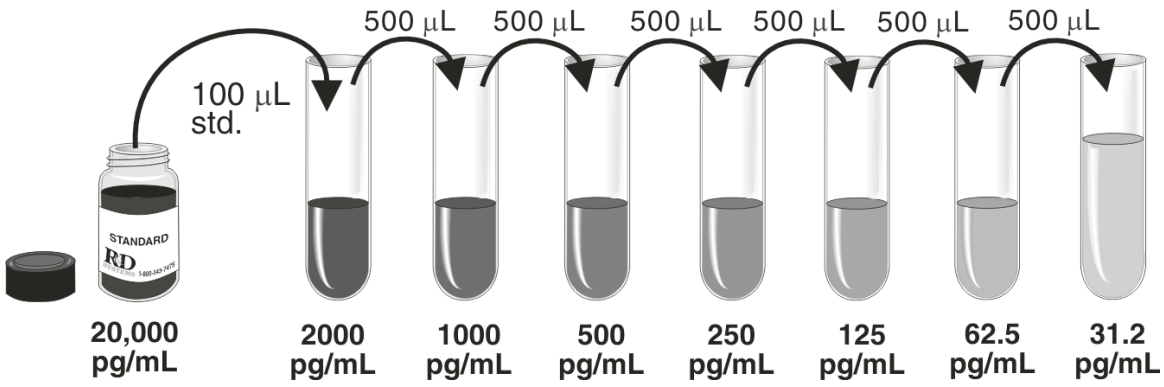
Note: *IL-16 is detectable in saliva and sweat. Take precautionary measures to prevent contamination of kit reagents while running the assay.*

Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Dilute 20 mL of Wash Buffer Concentrate into deionized or distilled water to prepare 500 mL of Wash Buffer.

Substrate Solution - Color Reagents A and B should be mixed together in equal volumes within 15 minutes of use. Protect from light. 200 µL of the resultant mixture is required per well.

IL-16 Standard - Reconstitute the IL-16 Standard with 1 mL of deionized or distilled water. This reconstitution produces a stock solution of 20,000 pg/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions.

Pipette 900 µL of Calibrator Diluent RD5R (*for cell culture supernate samples*) or Calibrator Diluent RD6-27 (*for serum/plasma samples*) into the 2000 pg/mL tube. Pipette 500 µL of the appropriate Calibrator Diluent into the remaining tubes. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The 2000 pg/mL standard serves as the high standard. The appropriate Calibrator Diluent 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 samples, standards, and controls be assayed in duplicate.

1. Prepare all reagents 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 100 µL of Assay Diluent RD1W to each well.

4. Add 100 µL of Standard, control, or sample per well. Cover with the adhesive strip provided. Incubate for 2 hours at room temperature. A plate layout is provided to record standards and samples assayed.

5. Aspirate each well and wash, repeating the process three times for a total of four 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 200 µL of IL-16 Conjugate to each well. Cover with a new adhesive strip. Incubate for 2 hours at room temperature.

7. Repeat the aspiration/wash as in step 5.

8. Add 200 µL of Substrate Solution to each well. Incubate for 30 minutes at room temperature. **Protect from light.**

9. Add 50 µL of Stop Solution to each well. The color in the wells should change from blue to yellow. If the color in the well is green or if the color change does not appear uniform, gently tap the plate to ensure thorough mixing.

10. 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.

ASSAY PROCEDURE SUMMARY

1. Prepare reagents, samples, and standards as instructed.

2. Add 100 µL Assay Diluent RD1W to each well.

3. Add 100 µL Standard, control, or sample to each well. Incubate 2 hours at RT.

4. Aspirate and wash 4 times.

5. Add 200 µL Conjugate to each well. Incubate 2 hours at RT.

6. Aspirate and wash 4 times.

7. Add 200 µL Substrate Solution to each well. Incubate 30 minutes at RT. **Protect from light.**

8. Add 50 µL Stop Solution to each well. Read at 450 nm within 30 minutes.
λ correction 540 or 570 nm

CALCULATION OF RESULTS

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

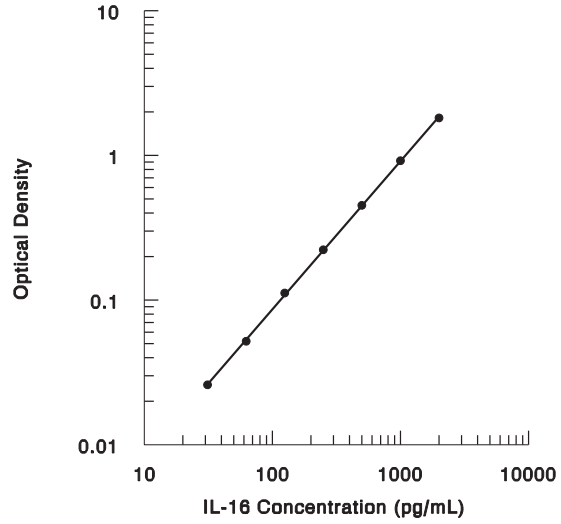
Create a standard curve by reducing the data using computer software capable of generating a log/log curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the log of the IL-16 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

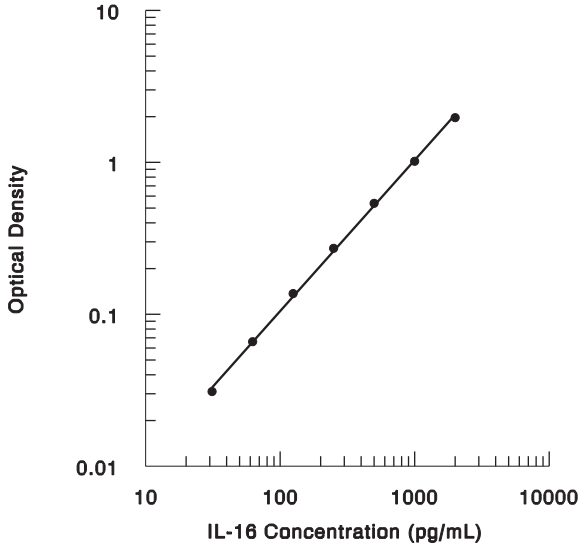
These standard curves are provided for demonstration only. A standard curve should be generated for each set of samples assayed.

Calibrator Diluent RD6-27



pg/mL	O.D.	Average	Corrected
0	0.068 0.067	0.068	—
31.2	0.093 0.093	0.093	0.025
62.5	0.123 0.118	0.120	0.052
125	0.183 0.178	0.180	0.112
250	0.297 0.283	0.290	0.222
500	0.544 0.495	0.520	0.452
1000	0.971 1.003	0.987	0.919
2000	1.931 1.842	1.886	1.818

Calibrator Diluent RD5R



pg/mL	O.D.	Average	Corrected
0	0.075 0.075	0.075	—
31.2	0.106 0.106	0.106	0.031
62.5	0.142 0.140	0.141	0.066
125	0.210 0.215	0.212	0.137
250	0.345 0.349	0.347	0.272
500	0.598 0.628	0.613	0.538
1000	1.078 1.107	1.092	1.017
2000	2.016 2.074	2.045	1.970

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.
- 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.
- To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
- 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.

PRECISION

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

Inter-assay Precision (Precision between assays)
Three samples of known concentration were tested in forty separate assays to assess inter-assay precision.

Serum/Plasma Assay

	Intra-assay Precision			Inter-assay Precision		
Sample	1	2	3	1	2	3
n	20	20	20	40	40	40
Mean (pg/mL)	241	747	1456	230	672	1315
Standard deviation	12.3	38.4	59.9	27.5	77.1	156
CV (%)	5.1	5.1	4.1	12.0	11.5	11.9

Cell Culture Supernate Assay

	Intra-assay Precision			Inter-assay Precision		
Sample	1	2	3	1	2	3
n	20	20	20	40	40	40
Mean (pg/mL)	205	615	1066	199	588	1135
Standard deviation	11.4	20.6	32.5	10.7	34.3	63.8
CV (%)	5.6	3.3	3.0	5.4	5.8	5.6

RECOVERY

The recovery of IL-16 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 = 4)	100	94 - 106%
Serum (n = 6)	103	94 - 113%
Heparin plasma (n = 6)	102	86 - 112%
EDTA plasma (n = 6)	105	96 - 117%

LINEARITY

To assess the linearity of the assay, samples containing or spiked with high concentrations of IL-16 were diluted with the appropriate Calibrator Diluent to produce samples with values within the dynamic range of the assay.

		Cell culture media (n = 7)	Serum* (n = 7)	Heparin plasma* (n = 7)	EDTA plasma* (n = 7)
1:2	Average % of Expected	102	102	100	102
	Range (%)	98 - 105	100 - 104	99 - 106	98 - 107
1:4	Average % of Expected	106	102	104	102
	Range (%)	100 - 113	98 - 110	98 - 115	95 - 106
1:8	Average % of Expected	100	100	100	101
	Range (%)	92 - 110	96 - 109	94 - 114	99 - 105
1:16	Average % of Expected	95	97	98	98
	Range (%)	87 - 107	92 - 111	91 - 109	92 - 102

*One sample containing natural levels of IL-16 was diluted 4-fold prior to assay.

SENSITIVITY

Forty-three assays were evaluated and the minimum detectable dose (MDD) of IL-16 ranged from 2.7 - 13.4 pg/mL. The mean MDD was 6.2 pg/mL.

The MDD was determined by adding two standard deviations to the mean optical density value of twenty zero standard replicates and calculating the corresponding concentration.

CALIBRATION

This immunoassay is calibrated against highly purified *E. coli*-expressed recombinant human IL-16 produced at R&D Systems.

SAMPLE VALUES

Serum/Plasma - Samples from apparently healthy volunteers were evaluated for the presence of IL-16 in this assay. No medical histories were available for the donors used in this study.

Sample Type	Mean (pg/mL)	Range (pg/mL)
Serum (n = 59)	171	77 - 280
Heparin plasma (n = 35)	122	62 - 206
EDTA plasma (n = 35)	204	71 - 480

One serum, one EDTA, and one heparin plasma sample measured 3817, 3473, and 3938 pg/mL respectively and were not included in the range.

Cell Culture Supernates - Human peripheral blood mononuclear cells (5 x 10^6 cells/mL) were cultured in RPMI supplemented with 5% fetal calf serum, 50 μM β-mercaptoethanol, 2 mM L-glutamine, 100 U/mL penicillin, and 100 μg/mL streptomycin sulfate. The cells were cultured unstimulated or stimulated with 10 μg/mL PHA. Aliquots of the cell culture supernate were removed on days 1 and 5 and assayed for levels of natural IL-16.

Condition	Day 1 (pg/mL)	Day 5 (pg/mL)
Unstimulated	143	638
Stimulated	482	1962

SPECIFICITY

This assay recognizes recombinant and natural human IL-16.The factors listed below were prepared at 50 ng/mL in Calibrator Diluent and assayed for cross-reactivity. Preparations of the following factors prepared at 50 ng/mL in a mid-range recombinant human IL-16 standard were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant human:

ANG
AR
sCD4
sCD8
CNTF
β-ECGF
EGF
Epo
FGF acidic
FGF basic
FGF-4
FGF-5
FGF-6
G-CSF
GM-CSF
sgp130
GROα
GROβ
GROγ
HB-EGF
HGF
IFN-γ
IGF-I
IGF-II
IL-1α
IL-1β
IL-1ra
IL-1 sRI
IL-1 sRII
IL-2
IL-2 sRα
IL-3
IL-3 sRα
IL-4
IL-4 sR
IL-5
IL-5 sRα
IL-5 sRβ
IL-6
IL-6 sR
IL-7
IL-8
IL-9
IL-10
IL-11
IL-12
IL-13
KGF (FGF-7)
LAP (TGF-β1)
LIF
M-CSF
MCP-1
MIP-1α
MIP-1β
β-NGF
OSM
PD-ECGF
PDGF-AA
PDGF-AB
PDGF-BB
PTN
RANTES
SCF
SLPI
TGF-α
TGF-β1
TGF-β2
TGF-β3
TGF-β sRII
TNF-α
TNF-β
sTNF RI
sTNF RII
VEGF

Recombinant mouse:

IL-1α
IL-1β
IL-3
IL-4
IL-5
IL-6
IL-7
IL-9
IL-10
IL-13
GM-CSF
LIF
MIP-1α
MIP-1β
SCF
TNF-α

Recombinant amphibian:

TGF-β5

Natural proteins:

bovine FGF acidic
bovine FGF basic
human PDGF
porcine PDGF
human TGF-β1
porcine TGF-β1

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PLATE LAYOUT

Use this plate layout to record standards and samples assayed.