Quantikine® ELISA

Human CCL17/TARC Immunoassay

Catalog Number DDN00
SDN00
PDDN00

For the quantitative determination of human Thymus and Activation-Regulated Chemokine (TARC) 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

TARC is a protein that was identified by cloning the D3A gene from peripheral blood mononuclear cells (PBMCs) after stimulation with PHA (1). The only other major source of expression was the thymus. Thus, the protein was named TARC for Thymus and Activation-Regulated Chemokine (1). TARC is a basic protein with a predicted mass of 8 kDa. It has 25-30% sequence homology with other known members of the CC chemokine family, including full conservation of the four cysteines that are characteristic of chemokines. TARC is most closely related to Macrophage-Derived Chemokine (MDC) (2).

TARC acts on the chemokine receptor CCR4, which is expressed on PBMCs and human T-cell lines but not on B cells, NK cells, or granulocytes (2, 3). MDC competes with TARC for binding to CCR4 (4). TARC also binds to CCR8 (5), which is expressed on lymphoid tissue. CCR4 and CCR8 expression is transiently up-regulated on activated T cells, preferentially on the Th2 subset (6). It thus appears that TARC is a chemokine specific for the trafficking of Th2 cells.

The Quantikine Human TARC Immunoassay kit is a 3.5 hour solid phase ELISA designed to measure TARC in cell culture supernates, serum, and plasma. It contains *E. coli*-expressed recombinant human TARC and antibodies raised against the recombinant factor. It has been shown to accurately quantitate recombinant human TARC. Results obtained using natural human TARC showed linear curves that were parallel to the standard curves obtained using the kit standards. These results indicate that this kit can be used to determine relative mass values for natural human TARC.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for TARC has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any TARC present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for TARC 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 TARC 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 generate values higher than the highest standard, 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.
- Variations in sample collection, processing, and storage may cause sample value differences.
- This assay is designed to eliminate interference by other factors present in biological samples. Until all factors have been tested in the Quantikine Immunoassay, the possibility of interference cannot be excluded.

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

PRECAUTIONS

Calibrator Diluent RD6Q 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 protective gloves, clothing, eye, and face protection. Wash hands thoroughly after handling.

MATERIALS PROVIDED & STORAGE CONDITIONS

Store the unopened kit at 2-8 °C. Do not use past kit expiration date.

PART	PART #	CATALOG # DDN00	CATALOG # SDN00	DESCRIPTION	STORAGE OF OPENED/ RECONSTITUTED MATERIAL
TARC Microplate	890076	1 plate	6 plates	96 well polystyrene microplate (12 strips of 8 wells) coated with a mouse monoclonal antibody against TARC.	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.*
TARC Conjugate	890077	1 vial	6 vials	21 mL/vial of polyclonal antibody against TARC conjugated to horseradish peroxidase with preservatives.	May be stored for up to 1 month at 2-8 °C.*
TARC Standard	890078	1 vial	6 vials	20 ng/vial of recombinant human TARC in a buffered protein base with preservatives; lyophilized.	
Assay Diluent RD1W	895117	1 vial	6 vials	11 mL/vial of a buffered protein base with preservatives.	
Calibrator Diluent RD5-5	895485	1 vial	6 vials	21 mL/vial of a buffered protein base with preservatives. *For cell culture supernate samples.*	
Calibrator Diluent RD6Q	895128	1 vial	6 vials	21 mL/vial of animal serum with preservatives. *For serum/plasma samples.*	
Wash Buffer Concentrate	895003	1 vial	6 vials	21 mL/vial of a 25-fold concentrated solution of buffered surfactant with preservative.	
Color Reagent A	895000	1 vial	6 vials	12 mL/vial of stabilized hydrogen peroxide.	
Color Reagent B	895001	1 vial	6 vials	12 mL/vial of stabilized chromogen (tetramethylbenzidine).	
Stop Solution	895032	1 vial	6 vials	6 mL/vial of 2 N sulfuric acid.	
Plate Sealers	N/A	4 strips	24 strips	Adhesive strips.	

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

DDN00 contains sufficient materials to run an ELISA on one 96 well plate.
SDN00 (SixPak) contains sufficient materials to run ELISAs on six 96 well plates.

This kit is also available in a PharmPak (R&D Systems, Catalog # PDDN00). PharmPaks contain sufficient materials to run ELISAs on 50 microplates. Specific vial counts of each component may vary. Please refer to the literature accompanying your order for specific vial counts.

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.
- **Polypropylene** test tubes for dilution of standards.
- Human TARC Controls (optional; available from R&D Systems).

SAMPLE COLLECTION & STORAGE

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

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 at room temperature 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 has not been validated for use in this assay.*
Hemolyzed samples are not suitable for use in this assay.

REAGENT PREPARATION

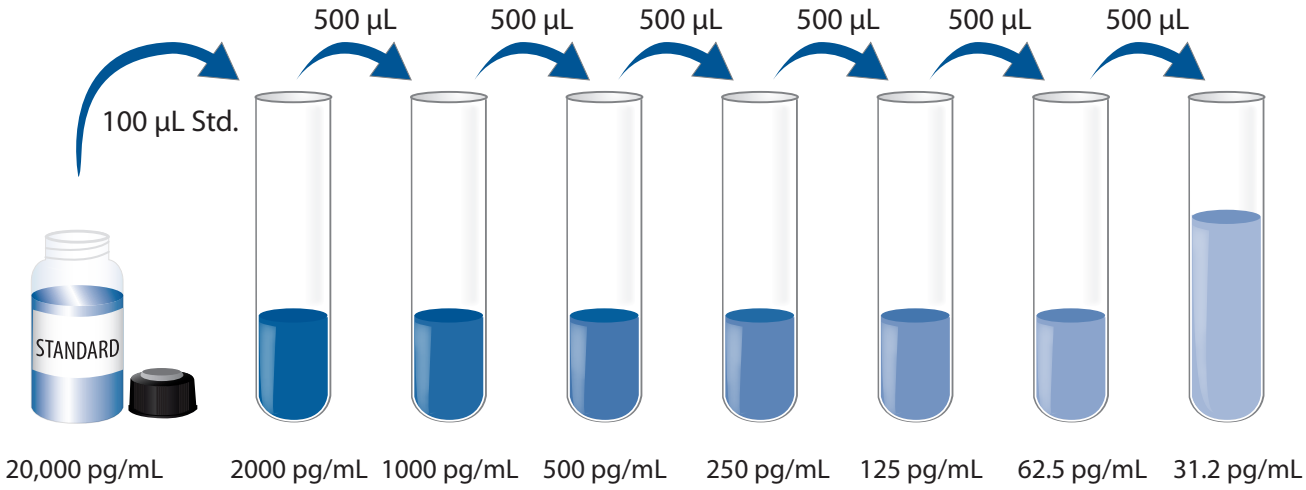
Bring all reagents to room temperature before use.

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.

TARC Standard - Reconstitute the TARC Standard with 1.0 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.

Use polypropylene tubes. Pipette 900 µL of Calibrator Diluent RD5-5 (*for cell culture supernate samples*) or Calibrator Diluent RD6Q (*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 standards, samples, and controls be assayed in duplicate.

1. Prepare all reagents, working standards, and samples 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 50 µL of Standard, control, or sample per well. Tap the plate gently for 1 minute to ensure adequate mixing. Cover with the adhesive strip provided. Incubate for 2 hours at room temperature.
5. Aspirate each well and wash, repeating the process two times for a total of three 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 toweling.
6. Add 200 µL of TARC Conjugate to each well. Cover with a new adhesive strip. Incubate for 1 hour 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. If color change does not appear uniform, gently tap the plate to ensure thorough mixing. If the color in the wells is green or 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.

CALCULATION OF RESULTS

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

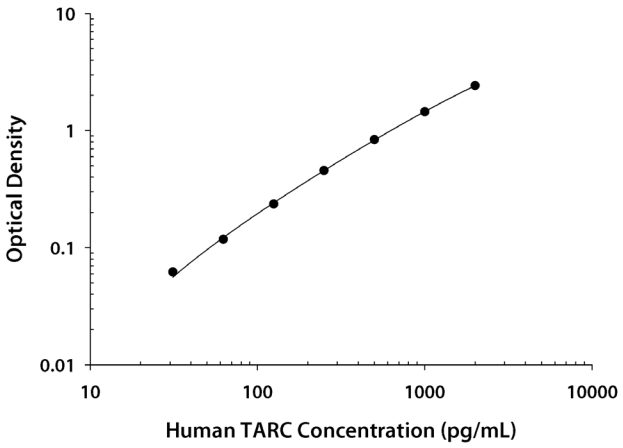
Create a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the log of the TARC 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

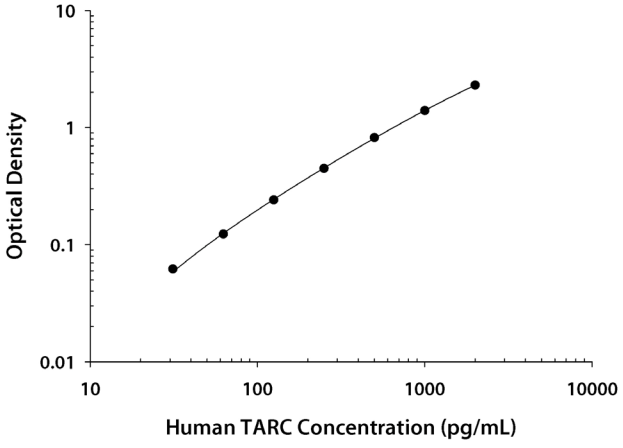
These standard curves are provided for demonstration only. A standard curve should be generated for each set of samples assayed.

CALIBRATOR DILUENT RD5-5



(pg/mL)	O.D.	Average	Corrected
0	0.037 0.035	0.036	—
31.2	0.099 0.097	0.098	0.062
62.5	0.154 0.154	0.154	0.118
125	0.269 0.274	0.272	0.236
250	0.483 0.500	0.492	0.456
500	0.876 0.873	0.874	0.838
1000	1.466 1.501	1.484	1.448
2000	2.420 2.492	2.456	2.420

CALIBRATOR DILUENT RD6Q



(pg/mL)	O.D.	Average	Corrected
0	0.024 0.024	0.024	—
31.2	0.085 0.087	0.086	0.062
62.5	0.148 0.146	0.147	0.123
125	0.264 0.266	0.265	0.241
250	0.469 0.475	0.472	0.448
500	0.846 0.845	0.845	0.821
1000	1.387 1.449	1.418	1.394
2000	2.357 2.293	2.325	2.301

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.

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)	88	504	1001	89	540	1033
Standard deviation	4.1	21.7	27.2	8.3	38.9	71.7
CV (%)	4.7	4.3	2.7	9.3	7.2	6.9

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)	87	600	1107	98	616	1178
Standard deviation	6.2	13.7	29.9	8.9	50.7	88.8
CV (%)	7.1	2.3	2.7	9.1	8.2	7.5

RECOVERY

The recovery of TARC spiked to levels throughout the range of the assay in various matrices was evaluated.

Sample Type	Average % Recovery	Range
Cell culture media (n=4)	95	88-104%
Serum (n=5)	105	100-114%
EDTA plasma (n=5)	102	86-111%
Heparin plasma (n=5)	98	86-107%

SENSITIVITY

The minimum detectable dose (MDD) of TARC is typically less than 7 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.

LINEARITY

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

		Cell culture media (n=6)	Serum (n=7)	EDTA plasma (n=5)	Heparin plasma (n=5)
1:2	Average % of Expected	107	105	103	104
	Range (%)	104-115	102-110	99-108	98-109
1:4	Average % of Expected	111	103	102	101
	Range (%)	107-115	96-112	93-112	95-112
1:8	Average % of Expected	110	102	100	101
	Range (%)	107-111	94-110	94-109	96-109
1:16	Average % of Expected	107	99	97	98
	Range (%)	104-110	92-110	92-105	91-106

CALIBRATION

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

SAMPLE VALUES

Serum/Plasma - Samples from apparently healthy volunteers were evaluated for the presence of TARC 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=66)*	331	71-848
EDTA plasma (n=34)*	134	35-285
Heparin plasma (n=35)	111	33-226

*Two serum samples and one EDTA plasma sample measured substantially higher and are not included in this range.

Cell Culture Supernates - Human peripheral blood mononuclear cells (5 x 10^6 cells/mL) were cultured in DMEM 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 supernates were removed on days 1 and 5 and assayed for levels of natural TARC.

Condition	Day 1 (pg/mL)	Day 5 (pg/mL)
Unstimulated	ND	1880
Stimulated	1336	1696

ND=Non-detectable

SPECIFICITY

This assay recognizes natural and recombinant human TARC.

The factors listed below were prepared at 50 ng/mL in Calibrator Diluent RD5-5 and Calibrator Diluent RD6Q and assayed for cross-reactivity. Preparations of the following factors prepared at 50 ng/mL in a mid-range recombinant human TARC control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant human:

ANG
AR
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
LAP (TGF-β1)
LIF
MCP-1
M-CSF
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:

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

Natural proteins:

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

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