Quantikine® ELISA

Human GCP-2 Immunoassay

Catalog Number DGC00

For the quantitative determination of human Granulocyte Chemotactic Protein 2 (GCP-2) 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.

TABLE OF CONTENTS

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INTRODUCTION

Human GCP-2 (granulocyte chemotactic protein-2, CXCL6), a CXC chemokine with a conserved Glu-Leu-Arg (ELR) motif, was originally isolated from the osteosarcoma cell line MG-63 as a 5-6 kDa protein (1). It is a potent neutrophil chemotactic and activating factor and exhibits extensive similarity to other CXC chemokines such as IL-8, GRO-α, and ENA-78 (for a review, see reference 2). GCP-2 can promote the release of MMP-9 from granulocytes indicating its potential role as an inflammatory mediator (3). The human GCP-2 gene has been cloned and is physically mapped to the CXC chemokine locus on chromosome 4 (4-6).

Mature human GCP-2 is a 75 amino acid (aa) protein with a predicted molecular weight of approximately 8 kDa (3, 4). Human GCP-2 shares 60% and 67% aa identity with mouse and bovine GCP-2, respectively. Among human chemokines, GCP-2 is most similar to ENA-78, having 77% aa homology. While three N-terminally truncated isoforms of the human protein have been isolated, none exhibit significant variation in activity from the mature protein (1). In contrast, N-terminally truncated forms of mouse GCP-2 are more active than the longer form, are more active than human GCP-2, and are equally active as human IL-8 on human neutrophils (7, 8). This observation, as well as the lack of a murine IL-8 homolog, has led to speculation that GCP-2 replaces IL-8 in the mouse as the major neutrophil-specific chemokine.

Like other ELR^+ CXC chemokines, GCP-2 activates target cells by binding to CXC chemokine receptor 1 (CXCR1) and CXCR2 (9-12). CXCR1 and CXCR2 are seven transmembrane-spanning, G-protein coupled receptors whose binding results in a rapid rise in cytosolic calcium concentration. Both receptors are expressed on neutrophils, but not on T- or B-lymphocytes.

GCP-2 promotes neutrophil migration and activation both *in vitro* and *in vivo* (1). Though maximal chemotactic response to GCP-2 is comparable to that induced by IL-8, about 10-fold less IL-8 is required for induction. Monocytes, eosinophils, and lymphocytes are all unresponsive to GCP-2 (2). GCP-2 may contribute to sepsis-associated tissue damage, hypersensitivity reactions, autoimmune conditions, and tumor invasion. In pregnant ruminants, GCP-2 expression is induced by IFN-τ and can be used as a marker to differentiate between IFN-τ and IFN-α induced responses (13, 14).

The Quantikine Human CXCL6/GCP-2 Immunoassay is a 4.5 hour solid-phase ELISA designed to measure human GCP-2 in cell culture supernates, serum, and plasma. It contains *E. coli*- expressed recombinant human GCP-2 and has been shown to accurately quantitate the recombinant factor. Results obtained using natural human GCP-2 showed linear curves that were parallel to the standard curves obtained using the Quantikine kit standards. These results indicate that this kit can be used to determine relative mass values for natural human GCP-2.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for human GCP-2 has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any GCP-2 present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for human GCP-2 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 GCP-2 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, further dilute the samples with 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.

MATERIALS PROVIDED & STORAGE CONDITIONS

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

PART	PART #	DESCRIPTION	STORAGE OF OPENED/ RECONSTITUTED MATERIAL
Human GCP-2 Microplate	890970	96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody specific for human GCP-2.	Return unused wells to the foil pouch containing the desiccant pack. Reseal along entire edge of the zip-seal. May be stored for up to 1 month at 2-8 °C.*
Human GCP-2 Conjugate	890971	21 mL of a polyclonal antibody specific for human GCP-2 conjugated to horseradish peroxidase with preservatives.	May be stored for up to 1 month at 2-8 °C.*
Human GCP-2 Standard	890972	Recombinant human GCP-2 in a buffered protein base with preservatives; lyophilized. *Refer to vial label for reconstitution volume.*	
Assay Diluent RD1W	895117	11 mL of a buffered protein base with preservatives.	
Calibrator Diluent RD5-24	895325	21 mL of a buffered protein base with preservatives.	
Wash Buffer Concentrate	895003	21 mL of a 25-fold concentrated solution of buffered surfactant with preservative. *May turn yellow over time.*	
Color Reagent A	895000	12 mL of stabilized hydrogen peroxide.	
Color Reagent B	895001	12 mL of stabilized chromogen (tetramethylbenzidine).	
Stop Solution	895032	6 mL of 2 N sulfuric acid.	
Plate Sealers	N/A	4 adhesive strips.	

* 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 of standards and samples.
- Human GCP-2 Controls (optional; R&D Systems, Catalog # QC23).

PRECAUTIONS

The Stop Solution provided with this kit is an acid solution.

Some components in this kit contain ProClin® which may cause an allergic skin reaction. Avoid breathing mist.

Color Reagent B may cause skin, eye, and respiratory irritation. Avoid breathing fumes.

Wear protective gloves, clothing, eye, and face protection. Wash hands thoroughly after handling. Please refer to the MSDS on our website prior to use.

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 heparin or EDTA 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.*
Grossly hemolyzed or lipemic samples are not suitable for use in this assay.

SAMPLE PREPARATION

Cell culture supernate samples require a 2-fold dilution. A suggested 2-fold dilution is 100 µL of sample + 100 µL of Calibrator Diluent RD5-24.

REAGENT PREPARATION

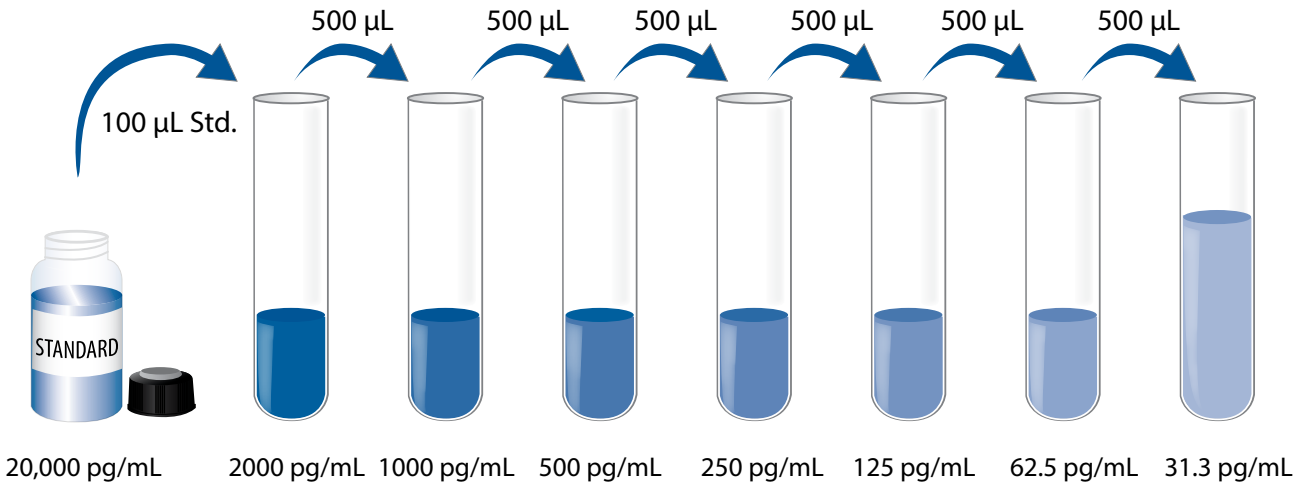
Bring all reagents to room temperature before use.

Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Add 20 mL of Wash Buffer Concentrate to 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.

Human GCP-2 Standard - **Refer to the vial label for reconstitution volume.** Reconstitute the Human GCP-2 Standard with deionized or distilled water. This reconstitution produces a stock solution of 20,000 pg/mL. Mix the standard to ensure complete reconstitution and allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions.

Pipette 900 µL of Calibrator Diluent RD5-24 into the 2000 pg/mL tube. Pipette 500 µL of Calibrator Diluent RD5-24 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 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. Cover with the adhesive strip provided. Incubate for 2 hours at room temperature.
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 Human GCP-2 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 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.

*Cell culture supernate samples require dilution. See Sample Preparation section.

CALCULATION OF RESULTS

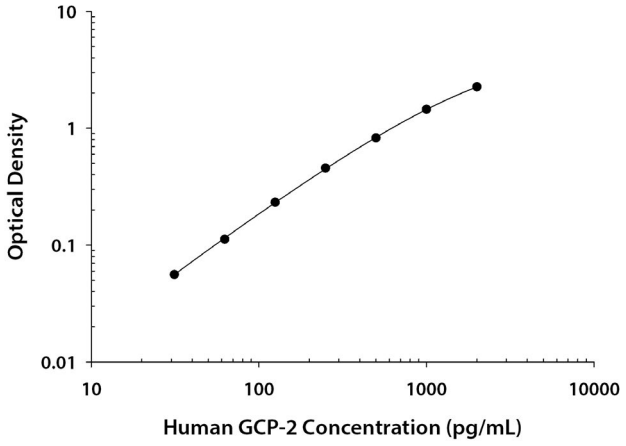
Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density (O.D.).

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 human GCP-2 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

This standard curve is provided for demonstration only. A standard curve should be generated for each set of samples assayed.



(pg/mL)	O.D.	Average	Corrected
0	0.011 0.009	0.010	—
31.3	0.069 0.062	0.066	0.056
62.5	0.117 0.128	0.122	0.112
125	0.243 0.241	0.242	0.232
250	0.475 0.457	0.466	0.456
500	0.857 0.816	0.836	0.826
1000	1.488 1.433	1.460	1.450
2000	2.292 2.237	2.264	2.254

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. Assays were performed by at least three technicians using two lots of components.

	Intra-Assay Precision			Inter-Assay Precision		
Sample	1	2	3	1	2	3
n	20	20	20	40	40	40
Mean (pg/mL)	273	517	1078	263	472	965
Standard deviation	13.7	30.5	57.7	20.7	35.5	65.6
CV (%)	5.0	5.9	5.4	7.9	7.5	6.8

RECOVERY

The recovery of human GCP-2 spiked to levels throughout the range of the assay in various matrices was evaluated.

Sample Type	Average % Recovery	Range
Cell culture media (n=4)	104	93-114%
Serum (n=5)	95	88-104%
EDTA plasma (n=5)	99	89-109%
Heparin plasma (n=5)	100	91-112%

LINEARITY

To assess the linearity of the assay, samples containing and/or spiked with high concentrations of human GCP-2 were serially diluted with Calibrator Diluent to produce samples with values within the dynamic range of the assay.

		Cell culture media (n=4)	MG-63 supernate* (n=1)	Serum (n=5)	EDTA plasma (n=5)	Heparin plasma (n=5)
1:2	Average % of Expected	100	95	102	104	103
	Range (%)	92-108	———	100-103	102-108	99-106
1:4	Average % of Expected	104	98	109	109	107
	Range (%)	95-114	———	101-113	102-114	103-111
1:8	Average % of Expected	101	93	106	108	105
	Range (%)	90-112	———	97-110	102-113	98-113
1:16	Average % of Expected	100	91	110	107	105
	Range (%)	88-114	———	101-115	96-113	98-110

*Samples were diluted prior to assay.

SENSITIVITY

Thirty-one assays were evaluated and the minimum detectable dose (MDD) of human GCP-2 ranged from 0.4-8.0 pg/mL. The mean MDD was 1.6 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 GCP-2 produced at R&D Systems.

SAMPLE VALUES

Serum/Plasma - Samples from apparently healthy volunteers were evaluated for the presence of human GCP-2 in this assay. No medical histories were available for the donors used in this study.

Sample Type	Mean (pg/mL)	Range (pg/mL)	Standard Deviation (pg/mL)
Serum (n=69)	157	60-357	58
EDTA plasma (n=34)	93	39-270	47
Heparin plasma (n=34)	136	52-282	57

One sample was considered an outlier. This sample measured 701, 451, and 481 pg/mL in serum, EDTA plasma, and heparin plasma, respectively.

Cell Culture Supernates:
Human peripheral blood mononuclear cells (1 x 10^6 cells/mL) were cultured in RPMI supplemented with 10% fetal calf serum, 10 ng/mL of recombinant human IL-2, and 50 µM β-mercaptoethanol. The cells were cultured unstimulated or stimulated with 10 µg/mL PHA. Aliquots of the culture supernates were removed on days 1 and 5 and assayed for levels of human GCP-2.

Condition	Day 1 (pg/mL)	Day 5 (pg/mL)
Unstimulated	ND	160
Stimulated	ND	ND

ND=Non-detectable

MG-63 human osteosarcoma cells were cultured in DMEM with 10% fetal bovine serum and grown to 100% confluence. An aliquot of the culture supernate was removed, assayed for human GCP-2, and measured 1758 pg/mL.

SPECIFICITY

This assay recognizes natural and recombinant human GCP-2.

The factors listed below were prepared at 50 ng/mL in Calibrator Diluent and assayed for cross-reactivity. Preparations of the following factors at 50 ng/mL in a mid-range GCP-2 control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant human:	Recombinant mouse:	Other recombinants:
BLC/BCA-1	BLC/BCA-1	porcine IL-8
GROα	CRG-2/IP-10	rat LIX
GROβ	GCP-2	
GROγ	KC	
IL-8	LIX	
IP-10	MIG	
I-TAC	SDF-1α	
MIG		
NAP-2		
SDF-1α		
SDF-1β		

Recombinant human ENA-78 cross-reacts approximately 0.3% in this assay.

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