Quantikine™ ELISA

Human Angiopoietin-1 Immunoassay

Catalog Number DANG10
SANG10
PDANG10

For the quantitative determination of human Angiopoietin-1 concentrations in cell culture supernates, serum, platelet-poor plasma, saliva, and human milk.

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

SECTION PAGE

Manufactured and Distributed by:

USA R&D Systems, Inc.
614 McKinley Place NE, Minneapolis, MN 55413
TEL: 800 343 7475 612 379 2956
FAX: 612 656 4400
E-MAIL: info@bio-techne.com

Distributed by:

Europe | Middle East | Africa Bio-Techne Ltd.
19 Barton Lane, Abingdon Science Park
Abingdon OX14 3NB, UK
TEL: +44 (0)1235 529449
FAX: +44 (0)1235 533420
E-MAIL: info.emea@bio-techne.com

China Bio-Techne China Co., Ltd.
Unit 1901, Tower 3, Raffles City Changning Office,
1193 Changning Road, Shanghai PRC 200051
TEL: +86 (21) 52380373 (400) 821-3475
FAX: +86 (21) 52371001
E-MAIL: info.cn@bio-techne.com

INTRODUCTION

Angiopoietin-1 (Ang-1) is a secreted glycoprotein best known for its putative roles in vascular development (1). It is a member of a family of growth factors that, in humans, also includes Ang-2 and Ang-4. Ang-3 appears to be the mouse counterpart to human Ang-4 (2). Ang-1 is a putative 498 amino acid (aa) protein with prominent structural features that includes an N-terminal coiled-coil domain and a C-terminal fibrinogen-like domain (3). The human and mouse proteins exhibit approximately 97% aa sequence identity (3). Ang-1 and Ang-2 exhibit approximately 60% aa sequence identity and both share the receptor tyrosine kinase Tie-2 (3, 4). Integrins may also mediate Ang-1 and Ang-2 activities (5). At least three alternatively spliced variants of Ang-1 exist, two of which are unable to activate Tie-2 and may act as endogenous suppressors of Ang-1 activity (6). Ang-1 is expressed widely in the mouse embryo and in adult human tissues, primarily by endothelial support cells, as well as megakaryocytes and platelets (4, 7). Ang-1 is thought to exist as a homo-trimer or in higher order homo-oligomeric complexes (8). It may also form heteromeric complexes with Ang-2 (8).

Ang-1 is a positive regulator of blood vessel development, remodeling, and maturation. It is a survival factor for endothelial cells (ECs) and supports the recruitment of ECs and peri-endothelial support cells (9-13). Ang-1/Tie-2-mediated cell survival may require the activity of PI3K/AKT signaling, while migration potentially involves several kinases including PI3K, FAK, and PAK (14-17). Ang-1/Tie-2-mediated sprouting is accompanied by the activation of PI3K, FAK, and secretion of the proteases Plasmin and MMP-2 (18).

In vivo, deletion of the Ang-1 gene is embryonic lethal and is accompanied by cardiac defects and a generalized decrease in vascular complexity (19). In addition, Ang-1 stimulates increased vascularization when over-expressed in brain and skin, an effect that may occur in synergy with VEGF (20-22). Although actions of the related family member Ang-2 on blood vessel growth are complex and context-dependent, it may act as a competitive inhibitor of Ang-1/Tie-2 activity under certain conditions (4, 23). Angiopoietin family activities are apparently not restricted to the blood vessel endothelia, as both Ang-1 and Ang-2 have possible involvement in the formation of the lymphatic vessels as well (23, 24).

In addition to its effects on vessel growth, Ang-1 has also been implicated in other biological processes. For instance, Ang-1/Tie-2 signaling may help maintain hematopoietic stem cells in a quiescent state within the bone marrow (25). It also enhances neutrophil and eosinophil adhesion and migration, and regulates blood vessel permeability (26-28). In the nervous system, Ang-1 may support neuronal growth, survival, and dendritic organization (22, 29, 30). Altered Ang-1 and Ang-2 levels have been implicated in tumor-associated angiogenesis (31). Studies have also shown increased levels of circulating Ang-1 associated with cancer and hypertension (32, 33).

The Quantikine™ Human Angiopoietin-1 Immunoassay is a 4.5 hour solid-phase ELISA designed to measure human Angiopoietin-1 in cell culture supernates, serum, platelet-poor plasma, saliva, and human milk. It contains NS0-expressed recombinant human Angiopoietin-1 and has been shown to accurately quantitate the recombinant factor. Results obtained using natural human Angiopoietin-1 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 Angiopoietin-1.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for human Angiopoietin-1 has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any Angiopoietin-1 present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked monoclonal antibody specific for human Angiopoietin-1 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 Angiopoietin-1 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 the appropriate calibrator diluent and repeat the assay.
- Any variation in 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 #	CATALOG # DANG10	CATALOG # SANG10	DESCRIPTION	STORAGE OF OPENED/ RECONSTITUTED MATERIAL
Human Angiopoietin-1 Microplate	892944	1 plate	6 plates	96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody specific for human Angiopoietin-1.	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 Angiopoietin-1 Standard	892946	1 vial	6 vials	Recombinant human Angiopoietin-1 in a buffered protein base with preservatives; lyophilized. *Refer to the vial label for reconstitution volume.*	May be stored for up to 1 month at -20 °C in a manual defrost freezer.*
Human Angiopoietin-1 Conjugate	892945	1 vial	6 vials	21 mL/vial of a monoclonal antibody specific for human Angiopoietin-1 conjugated to horseradish peroxidase with preservatives.	May be stored for up to 1 month at 2-8 °C.*
Assay Diluent RD1-20	895484	1 vial	6 vials	11 mL/vial of a buffered protein base with preservatives. *May contain a precipitate. Mix well before and during use.*	
Calibrator Diluent RD5P	895151	2 vials	12 vials	21 mL/vial of a buffered protein base with preservatives. *Use undiluted for serum/plasma samples. Use diluted 1:5 for cell culture supernate/saliva/human milk samples.*	
Wash Buffer Concentrate	895003	1 vial	6 vials	21 mL/vial of a 25-fold concentrated solution of buffered surfactant with preservative. *May turn yellow over time.*	
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 2N sulfuric acid.	
Plate Sealers	N/A	4 strips	24 strips	Adhesive strips.	

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

DANG10 contains sufficient materials to run an ELISA on one 96 well plate.
SANG10 (SixPak) contains sufficient materials to run ELISAs on six 96 well plates.

This kit is also available in a PharmPak (R&D Systems®, Catalog # PDANG10). Refer to the PharmPak Contents section for specific vial counts.

PHARMPAK CONTENTS

Each PharmPak contains reagents sufficient for the assay of 50 microplates (96 wells/plate). The package inserts supplied are the same as those supplied in the single kit packs and because of this, a few minor differences related to the number of reagents and their container sizes should be noted.

- Sufficient material is supplied to perform at least 50 standard curves; reuse of each vial may be required. The number of vials, and the number of standard curves obtained per vial will vary with the analyte.
- Wash Buffer 25X Concentrate is bulk packed in 125 mL bottles containing 100 mL.
  Note: *Additional wash buffer is available for purchase (R&D Systems®, Catalog # WA126).*

The reagents provided in this PharmPak are detailed below.

PART	PART #	QUANTITY
Human Angiopoietin-1 Microplate	892944	50 plates
Human Angiopoietin-1 Standard*	892946	50 vials
Human Angiopoietin-1 Conjugate	892945	50 vials
Assay Diluent RD1-20	895484	50 vials
Calibrator Diluent RD5P	895151	100 vials
Wash Buffer Concentrate	895126	9 bottles
Color Reagent A	895000	50 vials
Color Reagent B	895001	50 vials
Stop Solution	895032	50 vials
Plate Sealers	N/A	100 sheets
Package inserts	751413	2 booklets

**If additional standard vials are needed, contact Technical Service at techsupport@bio-techne.com*

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
- 100 mL and 500 mL graduated cylinders
- Horizontal orbital microplate shaker (0.12" orbit) capable of maintaining a speed of 500 ± 50 rpm
- **Polypropylene** test tubes for dilution of standards and samples
- Human Angiopoietin-1 Controls (optional; R&D Systems®, Catalog # QC42)

PRECAUTIONS

Angiopoietin-1 is detectable in saliva. Take precautionary measures to prevent contamination of kit reagents while running this assay.

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

Some components in this kit contain a preservative 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. Refer to the SDS 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.

Note: *Significant levels of Ang-1 are found in fetal bovine, bovine, porcine, equine, and rabbit sera. The background level of Ang-1 in control medium should be determined and subtracted from samples of conditioned media.*

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.

Platelet-poor Plasma - Collect plasma on ice using EDTA, heparin, or citrate as an anticoagulant. Centrifuge at 2-8 °C for 15 minutes at 1000 x g within 30 minutes of collection. For complete platelet removal, an additional centrifugation step of the separated plasma at 10,000 x g for 10 minutes at 2-8 °C is recommended. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Ang-1 is present in platelet granules and is released upon platelet activation. Therefore, to measure circulating levels of Ang-1, platelet-free plasma should be collected for measurement. It should be noted that many protocols for plasma preparation, including procedures recommended by the Clinical Laboratory and Standards Institute (CLSI), result in incomplete removal of platelets from blood. This will cause variable and irreproducible results for assays of factors contained in platelets and released by platelet activation.

Saliva - Collect saliva into a polypropylene tube. Centrifuge for 5 minutes at 10,000 x g in microcentrifuge tubes. Collect the aqueous layer (no pellet) and assay immediately or aliquot and store samples at 2-8 °C.

Human Milk - Centrifuge for 15 minutes at 1000 x g at 2-8 °C. Collect the aqueous fraction and repeat this process a total of 3 times. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

SAMPLE PREPARATION

Serum samples require a 50-fold dilution. A suggested 50-fold dilution is 10 µL of sample + 490 µL of Calibrator Diluent RD5P.

Platelet-poor plasma samples require a 15-fold dilution. A suggested 15-fold dilution is 10 µL of sample + 140 µL of Calibrator Diluent RD5P.

Saliva samples may require a 2-fold dilution. A suggested 2-fold dilution is 70 µL of sample + 70 µL of Calibrator Diluent RD5P (diluted 1:5)*.

Human milk samples require a 2-fold dilution. A suggested 2-fold dilution is 70 µL of sample + 70 µL of Calibrator Diluent RD5P (diluted 1:5)*.

*See Reagent Preparation section.

REAGENT PREPARATION

Bring all reagents to room temperature before use.

Note: *High concentrations of Angiopoietin-1 are found in saliva. It is recommended that a face mask and gloves are used to protect kit reagents from contamination.*

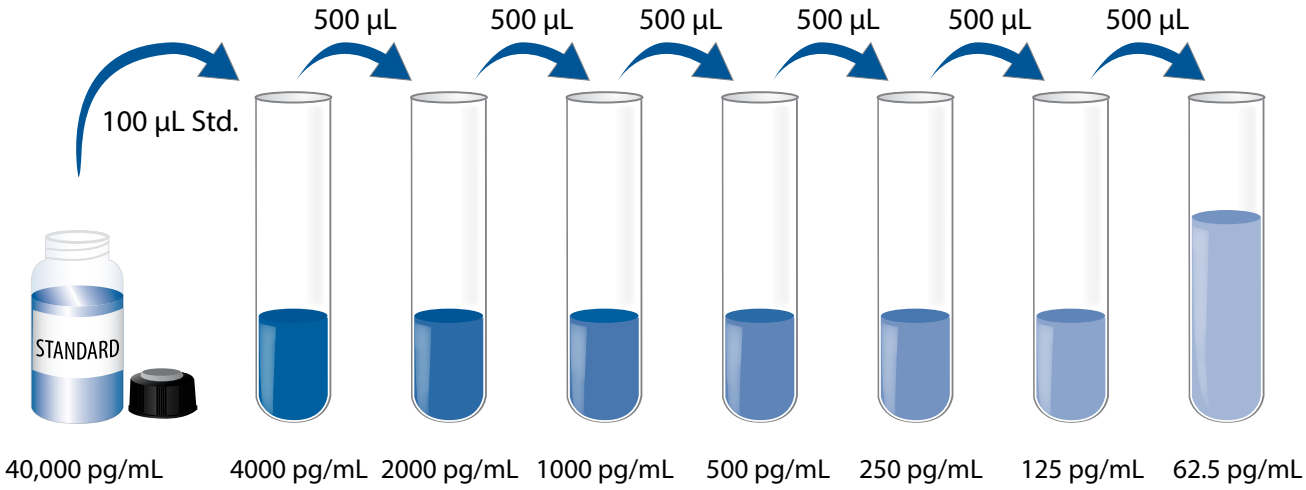
Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Add 20 mL of Wash Buffer Concentrate to 480 mL of deionized or distilled water to prepare 500 mL of Wash Buffer.

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.

Calibrator Diluent RD5P (diluted 1:5) - **For cell culture supernate/saliva/human milk samples only.** Add 20 mL of Calibrator Diluent RD5P to 80 mL of deionized or distilled water to prepare 100 mL of Calibrator Diluent RD5P (diluted 1:5).

Human Angiopoietin-1 Standard - **Refer to the vial label for reconstitution volume.** Reconstitute the Human Angiopoietin-1 Standard with deionized or distilled water. This reconstitution produces a stock solution of 40,000 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.

Use polypropylene tubes. Pipette 900 µL of Calibrator Diluent RD5P (diluted 1:5) (*for cell culture supernate/saliva/human milk samples*) or Calibrator Diluent RD5P (*for serum/plasma samples*) into the 4000 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 4000 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, controls, and samples be assayed in duplicate.

Note: *High concentrations of Angiopoietin-1 are found in saliva. It is recommended that a face mask and gloves are used to protect kit reagents from contamination.*

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 RD1-20 to each well. *Assay Diluent RD1-20 may contain a precipitate. Mix well before and during use.*
4. Add 50 µL of standard, control, or sample* per well. Cover with the adhesive strip provided. Incubate for 2 hours at room temperature on a horizontal orbital microplate shaker (0.12" orbit) set at 500 ± 50 rpm.
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 Angiopoietin-1 Conjugate to each well. Cover with a new adhesive strip. Incubate for 2 hours at room temperature on the shaker.
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 **on the benchtop. 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.

*Samples may 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.).

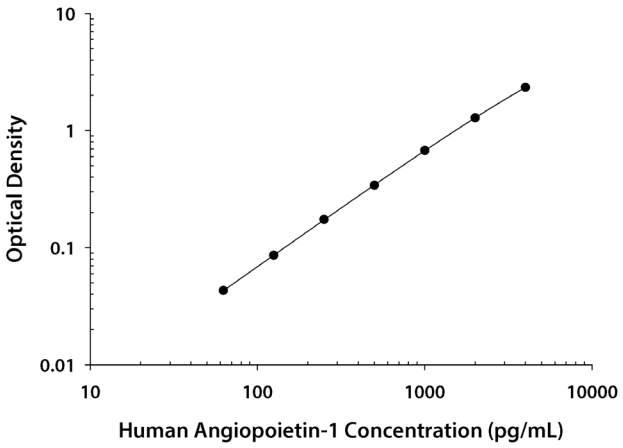
Create a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the log of the human Angiopoietin-1 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

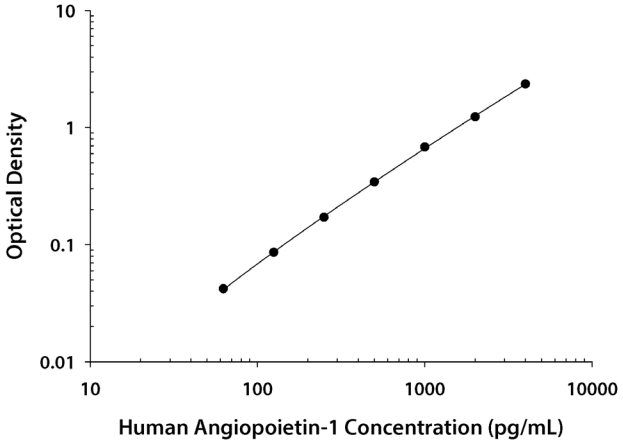
These standard curves are provided for demonstration only. A standard curve should be generated for each set of samples assayed.

CELL CULTURE SUPERNATE/SALIVA/HUMAN MILK ASSAY



(pg/mL)	O.D.	Average	Corrected
0	0.012 0.013	0.013	—
62.5	0.055 0.056	0.056	0.043
125	0.096 0.101	0.099	0.086
250	0.187 0.187	0.187	0.174
500	0.354 0.354	0.354	0.341
1000	0.684 0.691	0.688	0.675
2000	1.291 1.302	1.297	1.284
4000	2.336 2.359	2.348	2.335

SERUM/PLATELET-POOR PLASMA ASSAY



(pg/mL)	O.D.	Average	Corrected
0	0.013 0.013	0.013	—
62.5	0.053 0.056	0.055	0.042
125	0.096 0.101	0.099	0.086
250	0.179 0.188	0.184	0.171
500	0.347 0.367	0.357	0.344
1000	0.676 0.713	0.695	0.682
2000	1.210 1.281	1.246	1.233
4000	2.325 2.412	2.369	2.356

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.

CELL CULTURE SUPERNATE/SALIVA/HUMAN MILK ASSAY

	Intra-Assay Precision			Inter-Assay Precision		
Sample	1	2	3	1	2	3
n	20	20	20	40	40	40
Mean (pg/mL)	611	1217	2502	619	1223	2496
Standard deviation	20.8	24.2	67.0	42.4	71.5	113
CV (%)	3.4	2.0	2.7	6.8	5.8	4.5

SERUM/PLATELET-POOR 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)	587	1179	2339	620	1230	2533
Standard deviation	14.0	28.5	76.1	39.5	67.1	141
CV (%)	2.4	2.4	3.3	6.4	5.5	5.6

RECOVERY

The recovery of human Angiopoietin-1 spiked to levels throughout the range of the assay was evaluated.

Sample Type	Average % Recovery	Range
Cell culture media (n=4)	104	97-110%

LINEARITY

To assess the linearity of the assay, samples containing and/or spiked with high concentrations of human Angiopoietin-1 were serially diluted with the appropriate calibrator diluent to produce samples with values within the dynamic range of the assay.

		Cell culture media (n=4)	Serum* (n=4)	Platelet-poor plasma* (n=4)	Saliva (n=4)	Human milk* (n-3)
1:2	Average % of Expected	100	103	107	100	108
	Range (%)	98-101	97-106	99-114	95-102	98-114
1:4	Average % of Expected	100	93	99	100	113
	Range (%)	99-102	92-96	92-106	96-104	———
1:8	Average % of Expected	99	97	100	105	———
	Range (%)	99-100	93-101	93-108	103-106	———
1:16	Average % of Expected	96	94	100	114	———
	Range (%)	93-98	89-98	91-113	112-115	———

*Samples were diluted prior to assay as directed in the Sample Preparation section.

SENSITIVITY

One hundred assays were evaluated and the minimum detectable dose (MDD) of human Angiopoietin-1 ranged from 1.36-10.3 pg/mL. The mean MDD was 3.45 pg/mL.

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

CALIBRATION

This immunoassay is calibrated against a highly purified NS0-expressed recombinant human Angiopoietin-1 produced at R&D Systems®.

SAMPLE VALUES

Serum/Platelet-poor Plasma/Saliva/Human Milk - Samples from apparently healthy volunteers were evaluated for the presence of human Angiopoietin-1 in this assay. No medical histories were available for the donors used in this study. Samples were diluted prior to assay.

Sample Type	Mean of Detectable (pg/mL)	% Detectable	Range (pg/mL)
Serum (n=46)	37,122	100	14,272-65,570
Platelet-poor plasma (n=104)	ND	——	——
Saliva (n=10)	2641	100	972-4056
Human milk (n=4)	358	100	149-508

ND=Non-detectable

Cell Culture Supernates:

Note: *Significant levels of Ang-1 are found in fetal bovine, bovine, porcine, equine, and rabbit sera. The background level of Ang-1 in control medium should be determined and subtracted from samples of conditioned medium.*

Human peripheral blood mononuclear cells (5 x 10^6 cells/mL) were cultured in DMEM supplemented with 5% fetal bovine serum, 5 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 and assayed for levels of human Angiopoietin-1.

Condition	Day 1 (pg/mL)	Day 6 (pg/mL)
Unstimulated	351	250
Stimulated	309	108

HepG2 human hepatocellular carcinoma cells were cultured in DMEM supplemented with 5% fetal bovine serum until confluent and stimulated with 50 ng/mL PMA for 24 hours. An aliquot of the cell culture supernate was removed, assayed for human Angiopoietin-1, and measured 368 pg/mL.

A431 human epithelial carcinoma cells were cultured in DMEM supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin sulfate. An aliquot of the cell culture supernate was removed, assayed for human Angiopoietin-1, and measured 167 pg/mL.

MO7e human megakaryocytic leukemic cells were cultured in RPMI supplemented with 10% fetal bovine serum until confluent. Cells were cultured for 5-6 days, assayed for human Angiopoietin-1, and measured 259 pg/mL.

OVCAR-3 human ovarian carcinoma cells were cultured in RPMI supplemented with 20% fetal bovine serum, 10 µg/mL bovine insulin, 10 mM HEPES, 1 mM sodium pyruvate, 4.5 g/L glucose (total), and 1.5 g/L sodium bicarbonate until confluent. Cells were assayed for human Angiopoietin-1 and measured 694 pg/mL.

SPECIFICITY

This assay recognizes natural and recombinant human Angiopoietin-1.

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 recombinant human Angiopoietin-1 control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant human:

ANG
Ang-2
Ang-4
Ang-X
Ang-Y1
AR
BDNF
CD4
CNTF
CT-1
CTLA-4
β-ECGF
EGF
Epo
FGF acidic
FGF basic
FGF-4
FGF-5
FGF-6
FGF-9
FGF-10
FGF-18
Flt-3/Flk-2 Ligand
Flt-4
G-CSF
GDNF
GITR
GITR Ligand
GM-CSF
HB-EGF
HGF
HRG-α
IGF-I
IGF-II
IFN-α
KGF (FGF-7)
Leptin
LIF
M-CSF
MIF
MK
MSP
MSPβ
β-NGF
NT-3
NT-4
OPN
OSM
PD-ECGF
PDGF-AA
PDGF-AB
PDGF-BB
PlGF
PTN
SCF
SLPI
SMDF
Tie-1
Tie-2
TNF-α
TNF-β
Tpo
$VEGF_{121}$
$VEGF_{165}$
VEGF/PlGF
VEGF-D

Recombinant mouse:

CT-1
CTLA-4
FGF-8b
FGF-8c
Flt-3/Flk-2 Ligand
G-CSF
GM-CSF
IFN-α
Leptin
LIF
M-CSF
OPN
OSM
PlGF-2
SCF
Tie-2
TNF-α
Tpo
$VEGF_{120}$
$VEGF_{164}$

Recombinant rat:

CNTF
GDNF
GM-CSF
IFN-α
Leptin
β-NGF
PDGF-BB
TNF-α

Recombinant porcine:

GM-CSF
TNF-α

Recombinant zebrafish:

Tie-2

Natural proteins:

bovine FGF acidic
bovine FGF basic
human PDGF
porcine PDGF

REFERENCES

1. Tsigkos, S. *et al.* (2003) Expert Opin. Investig. Drugs **12**:933.
2. Valenzuela, D.M. *et al.* (1999) Proc. Natl. Acad. Sci. USA **96**:1904.
3. Davis, S. *et al.* (1996) Cell **87**:1161.
4. Maisonpierre, P.C. *et al.* (1997) Science **277**:55.
5. Dallabrida, S.M. *et al.* (2005) Circ. Res. **96**:e8.
6. Huang, Y.Q. *et al.* (2000) Blood **95**:1993.
7. Li, J.J. *et al.* (2001) Thromb. Haemost. **85**:204.
8. Procopio, W.N. *et al.* (1999) J. Biol. Chem. **274**:30196.
9. Witzenbichler, B. *et al.* (1998) J. Biol. Chem. **273**:18514.4.
10. Harfouche, R. *et al.* (2003) FASEB J. **17**:1523.
11. Velazquez, O.C. *et al.* (2002) FASEB J. **16**:1316.
12. Takakura, N. *et al.* (2000) Cell **102**:199.
13. Kwak, H.J. *et al.* (1999) FEBS Lett. **448**:249.
14. Master, Z. *et al.* (2001) EMBO J. **20**:5919.
15. Kontos, C.D. *et al.* (2002) Mol. Cell. Biol. **22**:1704.
16. Kim, I. *et al.* (2000) Circ. Res. **86**:24.
17. Jones, N. *et al.* (1999) J. Biol. Chem. **274**:30896.
18. Kim, I. *et al.* (2000) Circ. Res. **86**:952.
19. Suri, C. *et al.* (1996) Cell **87**:1171.
20. Suri, C. *et al.* (1998) Science **282**:468.
21. Thurston, G. (2002) J. Anat. **200**:575.
22. Ward, N.L. *et al.* (2005) J. Comp. Neurol. **482**:244.
23. Gale, N.W. *et al.* (2002) Dev. Cell **3**:411.
24. Morisada, T. *et al.* (2005) Blood **105**(12):4649.
25. Arai, F. *et al.* (2004) Cell **118**:149.
26. Lemieux, C. *et al.* (2005) Blood **105**:1523.
27. Thurston, G. *et al.* (2000) Nat. Med. **6**:460.
28. Feistritzer, C. *et al.* (2004) J. Allergy Clin. Immunol. **114**:1077.
29. Kosacka, J. *et al.* (2005) Cell Tissue Res. **320**:11.
30. Valable, S. *et al.* (2003) FASEB J. **17**:443.
31. Tait, C.R. and P.F. Jones. (2004) J. Pathol. **204**:1.
32. Nadar, S.K. *et al.* (2004) J. Intern. Med. **256**:331.
33. Caine, G.J. *et al.* (2003) Eur. J. Clin. Invest. **33**:883.15.