ANG2 ELISA

ANGIOPOIETIN-2

Cat.No. BI-ANG2
12x8 Tests

IMMUNOASSAY FOR THE QUANTITATIVE DETERMINATION OF HUMAN ANGIOPOIETIN-2 (ANG2) IN SERUM, EDTA PLASMA, HEPARIN PLASMA, AND CITRATE PLASMA

For research use only. Not for use in diagnostic procedures.

This kit was developed and manufactured by:

BIOMEDICA

This package insert must be read entirely before using this product.

Detailed information on the human Angiopoietin-2 ELISA, e.g. assay validation data, sample matrix comparisons, and stability data is available on our website.

Related Products

- Human VEGF ELISA (#BI-VEGF)
- Total soluble Neuropilin-1 ELISA (#BI-20409)
- Soluble Semaphorin 4D ELISA (#BI-20405)

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INTRODUCTION

ANGIOPOIETIN-2 PROTEIN and FUNCTION

Angiopoietin-2 (ANG2) is a 56.9 kDa glycosylated growth factor that is specific for endothelial cells (ECs). ANG2 is expressed in embryonic vessels and contributes to the formation of new vasculature. In adults, it is restricted to sites of vascular remodeling (e.g. ovary, uterus, placenta) and wound healing. ANG2 is regulated by the cytokine vascular endothelial growth factor (VEGF). Together with VEGF, ANG2 induces endothelial cell migration, proliferation, and vascular sprouting. During angiogenesis, ANG2 exerts its effects via the angiopoietin-1/TIE2 receptor signaling system on endothelial cells. Disruption of this signaling leads to the loss of endothelial integrity. In consequence, the endothelium responds to various pro-inflammatory cytokines and growth factors. Thus, ANG2 might cause vascular micro-inflammation in patients with chronic kidney disease (CKD). Various studies demonstrated that ANG2 levels increase with CKD stage and are associated with fluid overload and abnormal cardiac structure. Furthermore, ANG2 concentrations correlate with mortality in patients with CKD stages 4–5. Although ANG2 levels recover after successful kidney transplantation, ANG2 continues to be a cardiovascular risk factor in this population.

In cancer, targeting the TIE2-Angiopoietin pathway has shown promising results in some pre-clinical and clinical trials, including studies on recurrent or metastatic breast and renal cell carcinomas.

In COVID-19 patients, ANG2 was recently reported to be a relevant factor to predict transfer to the ICU as it was associated with poor lung compliance (13). Thus, showing that endothelial activation reinforces the hypothesis of a COVID-19-associated microvascular dysfunction. In this context, another study demonstrated that ANG2 levels in critically ill COVID-19 patients correlate with disease severity, hypercoagulation, and mortality. The researchers also provided novel in vivo evidence for a direct role for ANG2 in coagulation through binding to and inhibition of thrombomodulin-mediated anticoagulation. The scientists suggest that inhibition of ANG2 might be beneficial for treating critically ill COVID-19 patients, as well as other patients with hypercoagulation (14).

INTRODUCTION CONTINUED

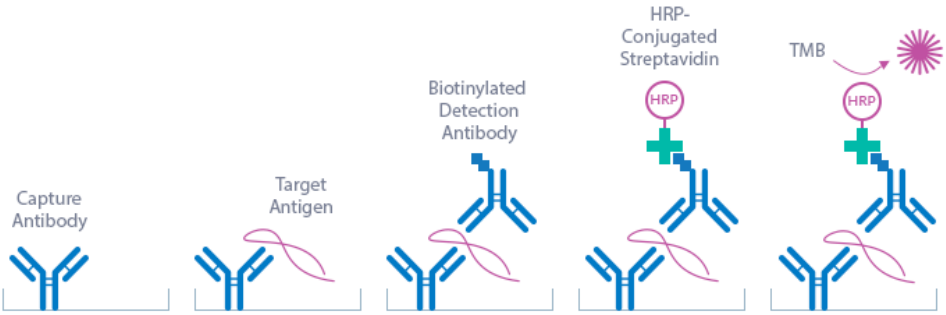
AREAS OF INTEREST

- Ischemic pathologies (PAD, CAD)
- Inflammation (Bowel disease, Chron's disease, cirrhosis, sepsis)
- Autoimmune disease (rheumatoid arthritis, psoriasis)
- Artherosclerosis
- Chronic kidney disease
- Diabetic retinopathy
- Cancer
- COVID-19

ASSAY PRINCIPLE

The Biomedica Angiopoietin-2 (ANG2) human ELISA kit is a sandwich enzyme immunoassay that has been optimized and fully validated for the quantitative determination of human Angiopoietin-2 in serum, EDTA plasma, heparin plasma and citrate plasma.s. Validation experiments have been performed according to international quality guidelines (ICH/ FDA/ EMEA). The ANG2 ELISA assay recognizes both natural and recombinant human ANG2. The assay employs highly purified epitope mapped antibodies as well as human serum-based standards and controls. Standards, controls and samples must be pre-diluted 1+10 prior to assaying.

The figure below explains the principle of the human Angiopoietin-2 sandwich ELISA:



In a first step, pre-diluted standard/control/sample and biotinylated antibody (goat polyclonal anti-human angiopoietin-2) are pipetted into the wells of the microtiter strips, which are pre-coated with a monoclonal anti-human angiopoietin-2 antibody. Angiopoietin-2 present in the standard/control/sample binds to the pre-coated antibody in the well and forms a sandwich with the anti-human angiopoietin-2 antibody. In a washing step all non-specific unbound material is removed. In a second step, the conjugate (streptavidin-HRPO) is pipetted into the wells and reacts with the biotinylated antibody. After another washing step, the substrate (TMB, tetramethylbenzidine) is pipetted into the wells.

The enzyme-catalyzed color change of the substrate is directly proportional to the amount of angiopoietin-2 present in the sample. This color change is detectable with a standard microplate reader. A dose response curve of the absorbance (optical density, OD at 450 nm) using the values obtained from the standards versus the standard concentration is generated. The concentration of angiopoietin-2 in the sample is determined directly from the dose response curve.

ELISA KIT COMPONENTS

All reagents supplied in the human ANG2 ELISA kit are stable at 2-8°C until the expiry date stated on the label of each reagent.

CONTENT	DESCRIPTION	QUANTITY
PLATE	Recombinant human monoclonal angiopoietin-2 antibody pre-coated microtiter strips in stripholder packed in aluminum bag with desiccant	12 x 8 tests
WASHBUF	20x wash buffer concentrate, transparent cap	1 x 50 ml
ASYBUF	Assay buffer, **red** cap, ready to use	1 x 22 ml
STD	Standards (0; 12.5; 25; 50; 100; 200; 400 pmol/l) recombinant human angiopoietin-2, white caps, lyophilized	7 vials
CTRL	Control A and B, human serum based, **yellow** cap, lyophilized, exact concentration is stated on labels	2 vials
AB	Goat polyclonal anti-human angiopoietin-2 antibody biotin-labeled, **green** cap, ready to use	1 x 7 ml
CONJ	Conjugate (streptavidin-HRPO), **brown** cap, ready to use	1 x 13 ml
SUB	Substrate (TMB solution), **blue** cap, ready to use	1 x 13 ml
STOP	STOP solution, white cap, ready to use	1 x 7 ml

ADDITIONAL KIT COMPONENTS

Two self-adhesive plastic films
Quality control protocol
Instruction for use
Plate layout sheet

OTHER SUPPLIES REQUIRED

Precision and multichannel pipettes calibrated to deliver 20 µl, 50 µl, 100 µl, 1000 µl, and disposable tips.
Distilled or deionized water.
A plate washer is recommended for washing. Alternatively use a multichannel pipette or manifold dispenser.
A microplate reader capable of measuring absorbance at 450nm (optionally with a correction wavelength at 630nm).
EP-tubes for sample dilution.
Software for the calculation of results or, alternatively, graph paper.
Refrigerator with 4°C (2-8°C).

SAMPLE COLLECTION AND STORAGE

Serum, EDTA plasma, heparin plasma, and citrate plasma are suitable for use in this assay. Do not change sample type during studies. The sample collection and storage conditions listed are intended as general guidelines.

SERUM & PLASMA

Collect venous blood samples by using standardized blood collection tubes. Perform plasma or serum separation by centrifugation according to supplier's instructions of the blood collection devices. Assay the acquired samples immediately or aliquot and store at -25°C or lower. Lipemic or haemolyzed samples may give erroneous results. Samples are stable for up to four freeze-thaw cycles. Samples should be mixed well before assaying. We recommend duplicates for all values. Samples with values above STD7 (400 pmol/l) can be diluted further with ASYBUF (Assay buffer).

Samples must be diluted 1+10 with assay buffer (ASYBUF), e.g. 20 µl sample + 200 µl ASYBUF.

REAGENT PREPARATION

WASH BUFFER

1.	Bring the WASHBUF concentrate to room temperature. Crystals in the buffer concentrate will dissolve at room temperature (18-26°C).
2.	Dilute the WASHBUF concentrate 1:20, e.g., 50 ml WASHBUF + 950 ml distilled or deionized water. Only use diluted WASHBUF when performing the assay.

The diluted WASHBUF is stable up to one month at 4°C (2-8°C).

STANDARDS & CONTROLS FOR SERUM AND PLASMA

1.	Pipette **200 µl** of distilled or deionized water into each standard (STD) and control (CTRL) vial. The exact concentration is printed on the label of each vial.
2.	Leave at room temperature (18-26°C) for 15 min. Vortex gently.

Reconstituted STDs and CTRL are stable at -25°C or lower until the expiry date stated on the label. STDs and CTRLs are stable for up to four freeze-thaw cycles.

STD / CTRL must be diluted 1+10 with assay buffer (ASYBUF), e.g. 20 µl STD / CTRL + 200 µl ASYBUF.

ASSAY PROTOCOL

Read the entire instructions for use before beginning the assay.

All reagents and samples must be at room temperature (18-26°C) before use in the assay.

Mix samples gently to ensure the samples are homogenous. We recommend performing duplicate measurements for all samples, standards and controls.

Mark position for STD/CTRL/SAMPLE (standard/control/sample) on the plate layout sheet.

Take microtiter strips out of the aluminum bag. Store unused strips with desiccant at 4°C (2-8°C) in the aluminum bag. Strips are stable until the expiry date stated on the label.

1.	Pipette 50 µl **1+10 pre-diluted** STD/CTRL/SAMPLE (standard/control/sample) in duplicate into respective wells. *See page 8, reagents and sample preparation, for pre-dilution.*
2.	Add **50 µl** AB (biotinylated anti-angiopoietin-2 antibody, **green** cap) into all wells, swirl gently.
3.	Cover tightly and incubate for **2 hours** at room temperature (18-26°C).
4.	Aspirate and wash wells 5x with 300 µl diluted WASHBUF (Wash buffer, transparent cap). After final wash, remove remaining WASHBUF by strongly tapping plate against paper towel.
5.	Add **100 µl** CONJ (Conjugate, **brown** cap) into each well. Swirl gently.
6.	Cover tightly and incubate for **1 hour** at room temperature (18-26°C).
7.	Aspirate and wash wells 5x with 300 µl diluted WASHBUF (Wash buffer, transparent cap). After final wash, remove remaining WASHBUF by strongly tapping plate against paper towel.
8.	Add **100 µl** SUB (Substrate, **blue** cap) into each well. Swirl gently.
9.	Incubate for **30 min** at room temperature (18-26°C), in the dark.
10	Add **50 µl** STOP (Stop solution, white cap) into each well. Swirl gently.
11.	Measure absorbance immediately at 450 nm with reference 630 nm, if available.

PRECAUTIONS

Do not pipette by mouth.
Do not eat, drink, smoke or apply cosmetics where reagents are used.
Refer to the Material Safety Data Sheet (MSDS) available for download at www.bmgrp.com.
All test components of human origin were tested against HIV-Ab, HCV-Ab, and HBsAg and were found negative. Nevertheless, they should be handled and disposed of as if they were infectious.
Avoid all contact with reagents by using protective gloves, clothing and eye protection.
Sulfuric acid contained in the STOP solution may cause irritations to eyes and skin. Avoid contact with skin and mucous membrane. – Flush with water if contact occurs!
Liquid reagents in this assay contain ≤0.1% Proclin 950 as a preservative. Proclin 950 is not toxic in concentrations used in this kit but may cause allergic skin reactions – avoid contact with skin or eyes.

TECHNICAL HINTS

Do not mix or substitute reagents with those from other lots or sources.
Do not mix stoppers and caps from different reagents or use reagents between lots.
Do not use reagents beyond the expiration date.
Protect reagents from direct sunlight.
Substrate solution should remain colorless until added to the plate.
Properly seal plates with the self-adhesive films during incubation steps to ensure accurate results.
Avoid foaming when mixing reagents.

CALCULATION OF RESULTS

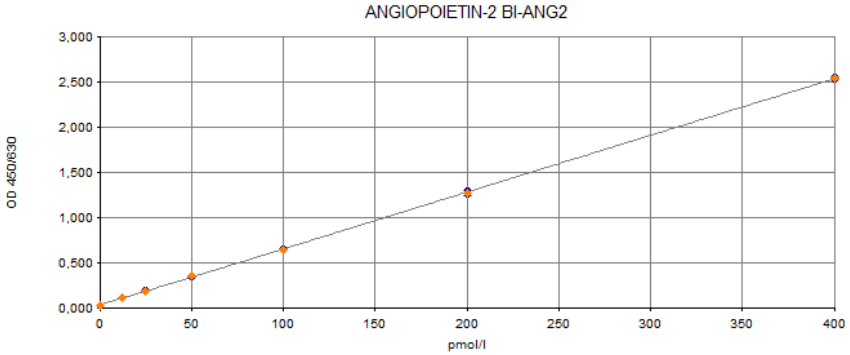
Construct a standard curve from the absorbance read-outs of the standards using commercially available software capable of generating a four-parameter logistic (4-PL) fit. Alternatively, plot the standards' concentration on the x-axis against the mean absorbance for each standard on the y-axis and draw a best fit curve through the points on the graph. Curve fitting algorithms other than 4-PL have not been validated and will need to be evaluated by the user.

Obtain sample concentrations from the standard curve. If required, the pmol/l concentration can be converted into pg/ml by applying a conversion factor: 1 pmol/l = 54.9 pg/ml (MW: 54.9 kDa).

Samples with analyte concentrations outside of the calibration range of the assay (400 pmol/l) should be diluted with assay buffer. **Respective dilution factors have to be considered when calculating the final concentration of the sample.**

TYPICAL DATA

This standard curve and the displayed OD values are for demonstration only. A standard curve should be generated for each assay run.



STANDARD	ANG2 pmol/l	OD			CV%
		#1	#2	AVERAGE	
STD1	0	0.035	0.034	0.035	2
STD2	12.5	0.119	0.122	0.121	2
STD3	25	0.205	0.190	0.198	5
STD4	50	0.365	0.355	0.360	2
STD5	100	0.653	0.660	0.657	1
STD6	200	1.303	1.261	1.282	2
STD7	400	2.536	2.559	2.548	1

The quality control protocol supplied with the kit shows the results of the final release QC for each kit at the production date. ODs obtained by customers may differ due to various influences including a normal decrease of signal intensity throughout shelf life. However, this does not affect the validity of the results provided an OD of 1.50 or higher is obtained for the standard with the highest concentration, and the measured control values fall into their target range (see labels).

ASSAY CHARACTERISTICS OVERVIEW

Method	Sandwich ELISA, HRPO/TMB, 12x8-well detachable strips			
Sample type(s)	Serum, EDTA plasma, citrate plasma, heparin plasma			
Sample volume	20 µl sample / well			
Standard range	0 – 400 pmol/l (0 / 12.5 / 25 / 50 / 100 / 200 / 400) (=0-21,960 pg/ml)			
Conversion factor	1 pmol/l= 54.9 pg/ml (MW: 54.9 kDa)			
Sensitivity	LOD: 3.7 pmol/l (=203 pg/ml); LLOQ: 6.3 pmol/l (=346 pg/ml)			
Assay time	2 h / 1 h / 30 min			
Precision		**n**	**Average % CV**	
	Within-run	3	≤8	
	In-between-run	9	≤6	
Accuracy (Spike/Recovery of recombinant human ANG2)		**n**	**Average % recovery**	
			+36 pmol/l	**+180 pmol/l**
	Serum	6	81	93
	Citrate plasma	1	100	95
		n	+40 pmol/l	+200 pmol/l
	EDTA plasma	6	85	78
	Heparin plasma	1	89	79
Parallelism of endogenous human ANG2		**n**	**Average % of expected dilution**	
			1+1	**1+3**
	Serum	6	105	105
	EDTA plasma	6	110	122
	Heparin plasma	1	107	109
	Citrate plasma	1	104	115
Specificity*	This assay recognizes recombinant and endogenous (natural) human Angiopoietin-2.			
Use	Research use only.			
Values of apparently healthy blood donors		**n**	**Median ANG2 (pmol/l)**	
	Serum	11	28	
	EDTA plasma	11	24	
	Heparin plasma	11	25	
	Citrate plasma	11	23	

**according to epitope mapping and sequence analysis the Angiopoietin-2 ELISA should detect all three Angiopoietin-2 isoforms. No cross-reactivity with Angiopoietin-1.*

PRECISION

WITHIN-RUN PRECISION

Within-run precision was tested by measuring two samples of known concentrations three times within one ANG2 ELISA lot by one operator.

IN-BETWEEN-RUN PRECISION

Within-run precision was tested by measuring two samples of known concentrations nine times within different ANG2 ELISA lots by different operators.

Within-run (n=3)	Sample 1	Sample 2	In-between-run (n=9)	Sample 1	Sample 2
Mean (pmol/l)	25	201	Mean (pmol/l)	26	201
SD (pmol/l)	2.1	3.0	SD (pmol/l)	1.6	5.1
CV (%)	8	1	CV (%)	6	3

SENSITIVITY

LOWER LIMIT OF DETECTION (LOD) & LOWER LIMIT OF QUANTIFICATION (LLOQ)

The LOD is defined as the mean back-calculated concentration of standard 1 (0 pmol/l of ANG2, five independent measurements) plus three times the standard deviation of the measurements.

The LLOQ is defined as the lowest concentration at which an analyte can be accurately quantified. To determine the LLOQ, standard 2, i.e., the lowest standard containing human ANG2 is diluted, measured five times and its concentration back calculated.

The following values were determined for the human ANG2 ELISA:

LOD	3.7 pmol/l
LLOQ	6.3 pmol/l

CALIBRATION

The human Angiopoietin-2 ELISA kit is calibrated against a highly purified recombinant human Angiopoietin-2 protein (Uniprot ID O15123, https://www.uniprot.org/uniprot/O15123).

The human serum based calibrator is provided in seven lyophilized glass vials in the following concentrations: 0 / 12.5 / 25 / 50 / 100 / 200 / 400 pmol/l (* = 0 / 686.25 / 1372.5 / 2745 / 5490 / 10980 / 21960 pg/ml).

*conversion factor: 1 pmol/l= 54.9 pg/ml (MW: 54.9 kDa)

SAMPLE VALUES

SPECIFICITY

This human ANG2 ELISA recognizes recombinant and endogenous (natural) human Angiopoietin-2.

According to epitope mapping and sequence analysis the Angiopoietin-2 ELISA should detect all three Angiopoietin-2 isoforms. No cross-reactivity with Angiopoietin-1.

CROSS REACTIVITY with non-human samples

This ELISA was tested in rat and porcine samples. According to our data the kit can be used for the detection of rat and porcine ANG2.

The sequence homology of human ANG2 to rat and porcine ANG2 sequence is 87% and 90%, respectively. We therefore analysed the presence of endogenous ANG2 signal in rat and porcine samples; the specificity was determined by competition.

Two rat samples and four pig samples were measured with this assay and showed a mean ANG2 concentration of 30 pmol/l for rat ANG2, and 62 pmol/l for porcine ANG2.
The obtained data for accuracy, parallelism, and competition experiments showed satisfactory results.

For more information please visit our website www.bmgrp.com.

Of note: Porcine samples were pre-diluted 1+10 and used according to assay protocol.

LITERATURE

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NOTES

NOTES

SYMBOLS

	Expiry date / Verfallsdatum / Date de péremption / Data di scadenza /Fecha de caducidad / Data de validade / Uiterste gebruiksdatum / Udløbsdato / Utgångsdatum / Termin Ważności / Lejárati idö / Doba exspirácie / Doba exspirace
	Consider instructions for use / Bitte Gebrauchsanweisung beachten / Consultez la notice d'utilisation / Consultare le istruzioni per l'uso / Consulte las instrucciones de utilización / Consulte as instruções de utilização / Raadpleeg de gebruiksaanwijzing / Se brugsanvisningen / Läs anvisningarna före användning / Proszę przeczytać instrukcję wykonania / Vegyük figyelembe a használati utasításban foglaltakat / Postupujte podl'a pokynov na použitie / Postupujte dle návodu k použití
	Lot-Batch Number / Charge-Chargennummer / Lot-Code du lot / Lotto-Numero di lotto / Lote-Código de lote / Lote-Código do lote / Lot-Partijnummer / Lot-Batchkode / Lot-Satskod / Numer serii / Lot-Batch szám / Číslo šarže / Číslo šarže
	Manufactured by / Hergestellt von / Fabriqué par / Prodotto da / Fabricado por / Fabricado por / Vervaardigd door / Fabrikation af / Tillverkad av / Wyprodukowane pr / Gyártotta / Vyrobené / Vyrobeno
	Catalogue Number / Bestellnummer / Numéro de référence / Numero di riferimento / Número de referencia / Número de referência / Referentienummer / Referencenummer / Katalognummer / Numer katalogowy / Katalógusszám / Katalógové číslo / Katalogové číslo
	Store at between / Lagerung bei zwischen / Conserver à entre / Conservare a tra / Conservar a temp. entre / Armazene a entre / Bewaar bij tussen / Opbevares mellem / Förvaras vid / Przechowywać w / Tároljuk ..... között / Skladujte v rozsahu / Skladujte v rozmezí
	Contains sufficient for x tests / Inhalt ausreichend für x Tests / Contient suffisant pour x tests / Contenuto sufficiente per x test / Contiene suficiente para x pruebas / Contém suficiente para x testes / Bevat voldoende voor x bepalingen / Indeholder tilstrækkeligt til x prøver / Innehållet räcker till x analyser / Zawartość na x testów / Tartalma X teszt elvégzésére elegendö / Obsahuje materiál pre x testov / Obsahuje materiál pro x testu

ASSAY PROTOCOL & CHECKLIST - FOR ALL SAMPLE TYPES

Human Angiopoietin-2 ELISA - # BI-ANG2

REAGENT PREPARATION

	Read the entire instruction for use before beginning the assay.
☐	Bring all reagents to room temperature (18-26°C).
☐	Prepare reagents and samples as instructed.
☐	Bring unused and prepared components to the storage temperature mentioned in the package insert.
☐	Take microtiter strips out of the aluminum bag and mark STD, CTRL, and SAMPLE positions on the plate layout sheet.

ASSAY PROCEDURE

☐	1. Pipette 50 µl **1+10 pre-diluted** STD/CTRL/SAMPLE (standard/control/sample) into respective wells.
☐	2. Add 50 µl AB (antibody, green cap) into each well, swirl gently.
☐	3. Cover tightly and **incubate for 2 hours** at room temperature (18-26°C).
☐	4. Aspirate and wash wells 5x with 300 µl diluted WASHBUF (Wash buffer, transparent cap). After final wash, remove remaining WASHBUF by strongly tapping plate against paper towel.
☐	5. Add **100 µl** CONJ (Conjugate, amber cap) into each well. Swirl gently.
☐	6. Cover tightly and **incubate for 1 hour** at room temperature (18-26°C).
☐	7. Aspirate and wash wells 5x with 300 µl diluted WASHBUF (Wash buffer, transparent cap). After final wash, remove remaining WASHBUF by strongly tapping plate against paper towel.
☐	8. Add **100 µl** SUB (Substrate, blue cap) into each well. Swirl gently.
☐	9. **Incubate for 30 min** at room temperature (18-26°C), **in the dark**
☐	10. Add **50 µl** STOP (Stop solution, white cap) into each well. Swirl gently.
☐	11. Measure absorbance immediately at 450 nm with reference 630 nm, if available.