# *ANGIOPOIETIN-2 MOUSE/RAT*

(EN) ELISA FOR THE QUANTITATIVE DETERMINATION OF MOUSE OR RAT ANGIOPOIETIN-2 IN SERUM OR PLASMA
Cat. No. BI-ANG2MR . 12 x 8 TESTS

FOR RESEARCH USE ONLY
NOT FOR USE IN DIAGNOSTIC PROCEDURES

rev.no. 191105

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## *CONTENT*



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## *1) INTRODUCTION*

Angiopoietin-2 (ANG2) is a 56.9 kDa glycosylated growth factor that is specific for endothelial cells (ECs) https://www.uniprot.org/uniprot/O35608 ANG2 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. Elevated ANG2 levels have also been observed in most cardiovascular disorders, such as coronary heart disease, congestive heart failure, and peripheral arterial disease. In terms of pathological angiogenesis, ANG2 has been widely explored in tumor-induced angiogenesis. The inhibition of ANG2 decreased the tumor size and metastatic efficacy. Thus, in cancer, targeting the TIE2-Angiopoietin pathway has shown promising results in some preclinical and clinical trials, including studies on recurrent or metastatic breast and renal cell carcinomas.

Areas of interest:

- Inflammation (Bowel disease, Chron's disease, cirrhosis, sepsis)
- Autoimmune disease (rheumatoid arthritis, psoriasis)
- Cardiovascular Disease
- Chronic kidney disease
- Cancer

## *2) CONTENTS OF THE KIT*

| CONT | KIT COMPONENTS | QUANTITY |
|---|---|---|
| PLATE | Detachable microtiter strips pre-coated with recombinant monoclonal Angiopoietin-2 antibody | 12 x 8 tests |
| WASHBUF | Wash buffer concentrate 20x, natural cap | 1 x 50 ml |
| ASYBUF | Assay buffer, red cap, ready to use | 1 x 15 ml |
| STOCK STD | Stock standard containing 1400 pmol/l of recombinant mouse Angiopoietin-2, red cap, lyophilised | 1 vial |
| CTRL | Control, yellow cap, lyophilised, exact concentration see label | 1 vial |
| AB | Polyclonal Angiopoietin-2 antibody, biotinylated, green cap, ready to use | 1 x 13 ml |
| CONJ | Streptavidin-HRPO conjugate, amber cap, ready to use | 1 x 13 ml |
| SUB | Substrate (TMB solution), blue cap, ready to use | 1 x 13 ml |
| STOP | STOP (Stop solution), white cap, ready to use | 1 x 7 ml |

## *3) ADDITIONAL MATERIAL IN THE KIT*

- 3 self-adhesive plastic films
- Quality control protocol
- Protocol sheet
- Instruction for use

## *4) MATERIAL AND EQUIPMENT REQUIRED BUT NOT SUPPLIED*

- Precision pipettes calibrated to deliver 5 µl, 50 µl, and 100 µl and disposable tips
- Distilled or deionised water
- Polypropylene tubes for standard curve preparation
- Plate washer is recommended for washing, alternative multichannel pipette or manifold dispenser
- Refrigerator with 4°C (2-8°C)
- ELISA reader capable of measuring absorbance at 450 nm (with correction wavelength at 630 nm)
- Graph paper or software for calculation of results

## *5) REAGENTS AND SAMPLE PREPARATION*

All reagents as supplied in the kit are stable at 4°C (2-8°C) until expiry date stated on the label of each reagent. Bring all reagents to room temperature before use.

Reconstituted STOCK STD and CTRL are stable at -25°C or lower until expiry date on label. STOCK STD and CTRL can undergo at least four freeze-thaw cycles.

**A. Sample preparation:**

Collect venous blood samples in 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. Samples are stable for up to four freeze-thaw cycles. Lipemic or haemolyzed samples may give erroneous results.
Samples with values above STD7 (1400 pmol/l) can be diluted with ASYBUF (Assay buffer).

Serum and plasma are suitable for use in this assay. Do not change sample type during studies.

**B. Preparation of CTRL (control):**

Reconstitute the CTRL (control) in 100 µl distilled or deionised water. Leave at room temperature (18-26°C) for 15 min and vortex gently prior to use. The exact concentration is stated on the label.

**C. Preparation of STOCK STD (stock standard):**

Reconstitute the mouse/rat Angiopoietin-2 STOCK STD (stock standard) in 100 µl distilled or deionised water. Leave at room temperature (18-26°C) for 15 min and vortex gently prior to use.

**D. Preparation of the standard curve**: *Always mix each tube thoroughly before the next step!*

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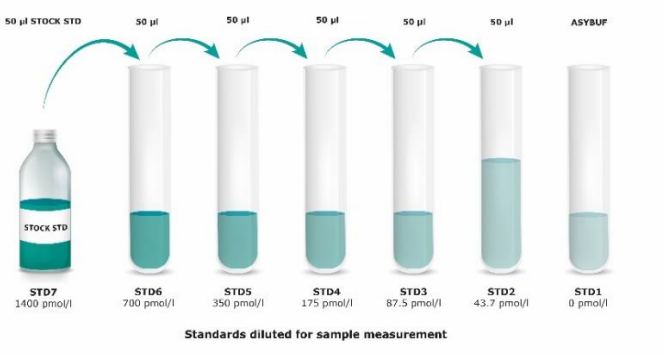
|---|
| 1. Use polypropylene tubes and mark them as STD6 to STD1 (Graph 1) |
| 2. Mark STOCK STD as STD7. |
| 3. Pipette 50 µl of ASYBUF (assay buffer) into each tube marked as STD6 to STD1. |
| 4. Prepare a two-fold serial dilution to obtain STD6 to STD2: Pipette 50 µl of the reconstituted STOCK STD (= STD7) into the tube labelled STD6. Mix thoroughly. Continue serial dilutions for STD5, STD4, STD3, STD2. |
| 5. ASYBUF serves as the zero standard (=STD1, 0 pmol/l). |

*Graph 1: Preparation of STD7 to STD1*

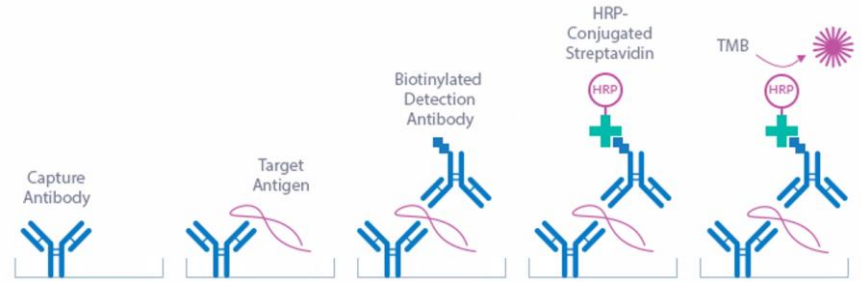


**Preparation of WASHBUF (wash buffer):**

Dilute the concentrate 1:20 (e.g. 50 ml WASHBUF + 950 ml distilled water). Crystals in the buffer concentrate will dissolve at room temperature (18-26°C). The undiluted WASHBUF is stable at 4°C (2-8°C) until expiry date stated on label. The diluted WASHBUF is stable up to one month at 4°C (2-8°C). Only use diluted WASHBUF when performing the assay.

## *6) PRINCIPLE OF THE ASSAY*

This kit is a sandwich enzyme immunoassay for the quantitative determination of mouse/rat Angiopoietin-2 in serum and plasma samples. In a first step, STD/CTRL/Sample are pipetted into the wells of the microtiter strips, which are pre-coated with a recombinant monoclonal Angiopoietin-2 antibody. Angiopoietin-2 present in the sample binds to the pre-coated antibody in the well. After a first wash step, which removes non-specifically unbound material, detection antibody is added and forms a sandwich with the antigen bound on the plate. After another washing step the conjugate (streptavidin-HRP) is pipetted into the wells and reacts with the detection antibody. After another washing step, the substrate (TMB, tetramethylbenzidine) is pipetted into the wells. The enzyme catalysed color change of the substrate is directly proportional to the amount of mouse/rat Angiopoietin-2 present in the sample. This color change is detectable with a standard microtiter plate ELISA reader. The concentration of Angiopoietin-2 in the sample is determined directly from the dose response curve.
The kit utilizes recombinant mouse Angiopoetin-2 as a calibrator. Mouse, rat, bovine and human Angiopoietin-2 share a high homology (>85%).




## *7) ASSAY PROTOCOL*

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| All reagents and samples must be at room temperature (18-26°C) before use in the assay. |
| Mark position for STD/CTRL/SAMPLE (Standard/Control/Sample) on the protocol sheet. |
| Take microtiter strips out of the aluminium bag. Store unused strips with desiccant at 4°C (2-8°C) in the aluminium bag. Strips are stable until expiry date stated on the label. |

| |
|---|
| 1) Pipette 100 µl ASYBUF (assay buffer, red cap) into each well. |
| 2) Add 5 µl STD/CTRL/SAMPLE in duplicates into the respective wells, swirl gently. |
| **3) Cover the plate tightly and incubate for 2 hours at room temperature (18-26°C) in the dark.** |
| 4) Aspirate and wash wells 5x with 300 µl diluted WASHBUF (wash buffer). After final wash, remove remaining WASHBUF by strongly tapping plate against paper towel. |
| 5) Add 100 µl AB (biotinylated anti-Angiopoietin-2 antibody, green cap) into each well, swirl gently. |
| **6) Cover the plate tightly and incubate for 2 hours at room temperature (18-26°C) in the dark.** |
| 7) Aspirate and wash wells 5x with 300 µl diluted WASHBUF (wash buffer). After final wash, remove remaining WASHBUF by strongly tapping plate against paper towel. |
| 8) Add 100 µl CONJ (conjugate, amber cap) into each well, swirl gently. |
| 9) **Cover the plate tightly and incubate for 1 hour at room temperature (18-26°C) in the dark.** |
| 10) Aspirate and wash wells 5x with 300 µl diluted WASHBUF (wash buffer). After final wash, remove remaining WASHBUF by strongly tapping plate against a paper towel. |
| 11) Add 100 µl SUB (substrate, blue cap) into each well, swirly gently. |
| **12) Incubate for 30 min at room temperature (18-26°C) in the dark.** |

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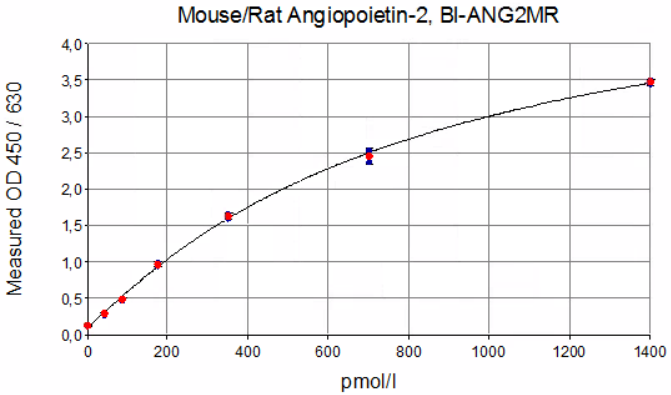
|---|
| 13) Add 50 µl STOP (stop solution, white cap) into each well, swirl gently. |
| 14) Measure absorbance immediately at 450 nm with reference 630 nm, if available. |

## *8) CALCULATION OF RESULTS*

Read the optical density (OD) of all wells on a plate reader using 450 nm wavelength (reference wavelength 630 nm). 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 need to be evaluated by the user. Obtain sample concentrations from the standard curve. If required, pmol/l can be converted into pg/ml by applying a conversion factor (1 pg/ml = 0.018 pmol/l; Mouse Angiopoietin-2 MW: 54.9 kDa). Respective dilution factors have to be considered when calculating the final concentration of the sample.

**Example typical STD-curve:**




The quality control (QC) protocol supplied with the kit shows the results of the final release QC for each kit lot. Data for OD obtained by customers may differ due to various influences and/or due to the normal decrease of signal intensity during shelf life. However, this does not affect validity of results as long as an OD of 1.00 or more is obtained for the STD with the highest concentration and the value of the CTRL is in range (target range see label).

## *9) ASSAY CHARACTERISTICS*

| | |
|---|---|
| **Method** | Sandwich ELISA, HRP/TMB, 12x8-well detachable strips |
| **Sample type** | Mouse or rat serum, plasma |
| **Standard range** | 0 – 1400 pmol/l (0 – 76,860 pg/ml) |
| **Conversion factor** | 1 pg/ml = 0.018 pmol/l (MW: 54.9 kDa) |
| **Sensitivity** | LOD: 18.3 pmol/l, LLOQ: 21.9 pmol/l |
| **Sample volume** | 5 µl / well |
| **Incubation time and temp.** | 2 h / 2 h / 1 h / 30 min, room temperature |
| **Specificity** | Endogenous and recombinant mouse/rat Angiopoietin-2. |
| **Precision** | Within-run (n=5) ≤ 4%, In-between-run (n=4) ≤ 9% |

| | | | | |
|---|---|---|---|---|
| **Accuracy** | | **Average % recovery** | | |
| | **Mouse (n=4)** | 101 | | |
| | **Rat (n=3)** | 100 | | |
| **Dilution linearity of endogenous Angiopoietin-2** | | **Average % of expected dilution** | | |
| | | **1+1** | **1+3** | **1+7** |
| | **Mouse (n=3)** | 100 | 102 | 101 |
| | **Rat (n=4)** | 92 | 94 | 104 |
| **Median Angiopoietin-2 values in various cohorts** | | **n** | **Angiopoietin-2 [pmol/l]** | |
| | **Mouse serum** | 18 | 105 | |
| | **Mouse plasma** | 7 | 127 | |
| | **Rat serum (clinical cohort)** | 8 | 292 | |
| | **Rat plasma** | 11 | 127 | |

For further information on assay and sample characteristics please visit our website www.bmgrp.com or contact our customer service by e-mail info@bmgrp.com or by phone +43/ 1/ 29107-45.

## *10) PRECISION*

Within-run (intra-assay): 2 samples of known concentrations were tested 5 times within 1 kit lot by 1 operator.

In-between-run (inter-assay): 2 samples of known concentrations were tested 4 times within 2 kit lots by 2 different operators.

| Intra-assay (n= 5) | Sample 1 | Sample 2 |
|---|---|---|
| Mean (pmol/l) | 77 | 120 |
| SD (pmol/l) | 3.0 | 2.2 |
| CV (%) | 4 | 2 |

| Inter-assay (n= 4) | Sample 1 | Sample 2 |
|---|---|---|
| Mean (pmol/l) | 144 | 182 |
| SD (pmol/l) | 4.5 | 16.6 |
| CV (%) | 3 | 9 |

Detailed information on the Angiopoietin-2 Mouse/Rat ELISA, e.g. assay performance characteristics is available on our website www.bmgrp.com .

## *11) 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 expiration date.
- Protect reagents from direct sunlight.
- Substrate solution should remain colourless until added to the plate.
- To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
- Avoid foaming when mixing reagents.

## *12) PRECAUTIONS*

All liquid reagents contain ≤ 0.1% Proclin 950 as preservative. Avoid contact with skin and mucous membrane. Proclin 950 is not toxic in concentrations used in this kit. It may cause allergic skin reactions – avoid contact with skin or eyes.

- Do not pipette by mouth.
- Do not eat, drink, smoke, or apply cosmetics where reagents are used.
- Wear gloves, glasses, and lab coat while performing this assay.
- Sulfuric acid is irritating to the eyes and skin. Avoid contact with skin and mucous. Irritations are possible. Flush with water if contact occurs.

## *13) LITERATUR*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4. Angiopoietin-2 serum levels correlate with severity, early onset and cardiovascular disease in patients with rheumatoid arthritis. López-Mejías R et al., Clin Exp Rheumatol, 2013; 31(5):761-6.
5. Serum angiopoietin-2 level as a potential biomarker in psoriasis vulgaris. Takahashi T et al., J Dermatol 2017; 44(2):205-206.
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8. Circulating Angiopoietin-2 levels predict mortality in kidney transplant recipients: a 4-year prospective case-cohort study. Molnar MZ et al., Transpl Int, 2014; 27(6):541–52.
9. The interaction between fluid status and angiopoietin-2 in adverse renal outcomes of chronic kidney disease. Tsai YC et al., PLoS One, 2017; 12 (3): e173906
10. Biomarker Profiling in Stage 5 Chronic Kidney Disease Identifies the Relationship between Angiopoietin-2 and Atrial Fibrillation. Bontekoe J et al., Clin Appl Thromb Hemost, 2018; doi: 10.1177/1076029618808909. [Epub ahead of print].
11. Angiopoietin-2, Angiopoietin-1 and subclinical cardiovascular disease in Chronic Kidney Disease. Tsai YC et al., Scientific Reports, 2016; 6:39400.
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14. Multiple effects of angiopoietin-2 blockade on tumors. Lewis CE and N Ferrara, Cancer Cell 2011, 12;19(4):431-3.
15. Inhibition of VEGF and Angiopoietin-2 to Reduce Brain Metastases of Breast Cancer Burden. Bohn KA et al., Front Pharmacol, 2017; 11;8:193.

# SYMBOLS

Expiry date / Verfallsdatum / Date de péremption / Data di scadenza /Fecha de caducidad / Data de validade / Uiterste gebruiksdatum / Udløbsdato / Utgångsdatum / Termin Ważności / Lejárati idő / 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 podľa 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 elegendő / Obsahuje materiál pre x testov / Obsahuje materiál pro x testů

# *# BI-ANG2MR ANGIOPOIETIN-2 MOUSE/RAT*
## *ASSAY PROTOCOL AND CHECKLIST*

***PREPARATION OF REAGENTS:***

- ❑ Bring all reagents to room temperature (18-26°C).
- ❑ Prepare reagents and samples as instructed.
- ❑ Take microtiter strips out of the aluminium bag and mark positions on the protocol sheet.
- ❑ Bring unused components to the storage temperature mentioned in the package insert.

***TEST PROCEDURE:***

- ❑ Step 1) Pipette 100 µl ASYBUF (assay buffer, red cap) into each well.
- ❑ Step 2) Add 5 µl STD/CTRL/SAMPLE in duplicates into the respective wells, swirl gently.
- ❑ **Step 3) Cover the plate tightly and incubate for 2 hours at room temperature (18-26°C) in the dark.**
- ❑ Step 4) Aspirate and wash wells 5x with 300 µl diluted WASHBUF (wash buffer). After the final wash, remove the remaining WASHBUF by strongly tapping plate against a paper towel.
- ❑ Step 5) Add 100 µl AB (biotinylated anti-Angiopoietin-2 antibody, green cap) into each well, swirl gently.
- ❑ **Step 6) Cover the plate tightly and incubate for 2 hours at room temperature (18-26°C) in the dark.**
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- ❑ Step 8) Add 100 µl CONJ (conjugate, amber cap) into each well, swirl gently.
- .❑ Step 9) **Cover tightly and incubate for 1 hour at room temperature (18-26°C) in the dark.**
- ❑ Step 10) Aspirate and wash wells 5x with 300 µl diluted WASHBUF (wash buffer). After the final wash, remove remaining WASHBUF by strongly tapping plate against a paper towel.
- ❑ Step 11) Add 100 µl SUB (substrate, blue cap) into each well, swirl gently.
- ❑ Step 12) **Incubate for 30 min at room temperature (18-26°C) in the dark.**
- ❑ Step 13) Add 50 µl STOP (stop solution, white cap) into each well, swirl gently.
- ❑ Step 14) Measure absorbance immediately at 450 nm with reference 630 nm, if available.