

Datasheet for ABIN1979481  
**Angiopoietin 4 ELISA Kit**[Go to Product page](#)

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## Overview

Quantity: 96 tests

Target: Angiopoietin 4 (ANGPT4)

Reactivity: Human

Method Type: Sandwich ELISA

Detection Range: 0.25-200 ng/mL

Minimum Detection Limit: 0.25 ng/mL

Application: ELISA

## Product Details

Purpose: Human Angiopoietin-4 ELISA Kit for cell culture supernatants, plasma, and serum samples.

Sample Type: Plasma, Cell Culture Supernatant, Serum

Analytical Method: Quantitative

Detection Method: Colorimetric

Specificity: This ELISA antibody pair detects human Angiopoietin-4. Other species not determined.

Characteristics:

- Strip plates and additional reagents allow for use in multiple experiments
- Quantitative protein detection
- Establishes normal range
- The best products for confirmation of antibody array data

Components:

- Pre-Coated 96-well Strip Microplate
- Wash Buffer
- Stop Solution

## Product Details

- Assay Diluent(s)
- Lyophilized Standard
- Biotinylated Detection Antibody
- Streptavidin-Conjugated HRP
- TMB One-Step Substrate

### Material not included:

- Distilled or deionized water
- Precision pipettes to deliver 2 µL to 1 µL volumes
- Adjustable 1-25 µL pipettes for reagent preparation
- 100 µL and 1 liter graduated cylinders
- Tubes to prepare standard and sample dilutions
- Absorbent paper
- Microplate reader capable of measuring absorbance at 450nm
- Log-log graph paper or computer and software for ELISA data analysis

## Target Details

Target:	Angiopietin 4 (ANGPT4)
Alternative Name:	Angiopietin-4 ( <a href="#">ANGPT4 Products</a> )
Gene ID:	51378
UniProt:	<a href="#">Q9Y264</a>
Pathways:	<a href="#">RTK Signaling</a>

## Application Details

Application Notes:	Recommended Dilution for serum and plasma samples2 fold
Sample Volume:	100 µL
Plate:	Pre-coated
Protocol:	<ol style="list-style-type: none"><li>1. Prepare all reagents, samples and standards as instructed in the manual.</li><li>2. Add 100 µL of standard or sample to each well.</li><li>3. Incubate 2.5 h at RT or O/N at 4 °C.</li><li>4. Add 100 µL of prepared biotin antibody to each well.</li><li>5. Incubate 1 h at RT.</li><li>6. Add 100 µL of prepared Streptavidin solution to each well.</li><li>7. Incubate 45 min at RT.</li><li>8. Add 100 µL of TMB One-Step Substrate Reagent to each well.</li><li>9. Incubate 30 min at RT.</li><li>10. Add 50 µL of Stop Solution to each well.</li></ol>

11. Read at 450 nm immediately.

Reagent Preparation:	<p>1. Bring all reagents and samples to room temperature (18 - 25 °C) before use. 2. Sample dilution: If your samples need to be diluted, Assay Diluent A (Item D) should be used for dilution of serum/plasma samples. 1x Assay The Human Angiopoietin-4 ELISA Kit Protocol 3 Diluent B (Item E) should be used for dilution of culture supernatants and urine. Suggested dilution for normal serum/plasma: 2 fold*. * Please note that levels of the target protein may vary between different specimens. Optimal dilution factors for each sample must be determined by the investigator. 3. Assay Diluent B should be diluted 5-fold with deionized or distilled water before use. 4. Preparation of standard: Briefly spin the vial of Item C and then add 400 µL Assay Diluent A (for serum/plasma samples) or 1x Assay Diluent B (for cell culture supernates/urine) into Item C vial to prepare a 200 ng/ml standard. Dissolve the powder thoroughly by a gentle mix. Pipette 400 µL Assay Diluent A or 1x Assay Diluent B into each tube. Use the 200 ng/ml standard solution to produce a dilution series (shown below). Mix each tube thoroughly before the next transfer. Assay Diluent A or 1x Assay Diluent B serves as the zero standard (0 ng/ml).</p> <table><tr><td>200 µL</td><td>200 µL</td><td>200 µL</td><td>200 µL</td><td>200 µL</td><td>200µL</td></tr><tr><td>Standard, Item C + 400 µL</td><td>200</td><td>66.67</td><td>22.22</td><td>7.41</td><td></td></tr><tr><td></td><td>2.47</td><td>0.823</td><td>0.274</td><td>0</td><td>ng/ml</td></tr><tr><td></td><td>ng/ml</td><td>ng/ml</td><td>ng/ml</td><td>ng/ml</td><td>ng/ml</td></tr><tr><td></td><td>ng/ml</td><td>ng/ml</td><td>ng/ml</td><td>ng/ml</td><td>ng/ml</td></tr></table> <p>5. If the Wash Concentrate (20x) (Item B) contains visible crystals, warm to room temperature and mix gently until dissolved. Dilute 20 mL of Wash Buffer Concentrate into deionized or distilled water to yield 400 mL of 1x Wash Buffer. The Human Angiopoietin-4 ELISA Kit Protocol 4 6. Briefly spin the Detection Antibody vial (Item F) before use. Add 100 µL of 1x Assay Diluent B into the vial to prepare a detection antibody concentrate. Pipette up and down to mix gently (the concentrate can be stored at 4 °C for 5 days). The detection antibody concentrate should be diluted 80-fold with 1x Assay Diluent B and used in step 4 of Part VI Assay Procedure. 7. Briefly spin the HRP-Streptavidin concentrate vial (Item G) and pipette up and down to mix gently before use. HRP-Streptavidin concentrate should be diluted 600-fold with 1x Assay Diluent B. For example: Briefly spin the vial (Item G) and pipette up and down to mix gently . Add 20 µL of HRP-Streptavidin concentrate into a tube with 12 mL 1x Assay Diluent B to prepare a 600-fold diluted HRP- Streptavidin solution (don't store the diluted solution for next day use). Mix well.</p>	200 µL	200 µL	200 µL	200 µL	200 µL	200µL	Standard, Item C + 400 µL	200	66.67	22.22	7.41			2.47	0.823	0.274	0	ng/ml		ng/ml	ng/ml	ng/ml	ng/ml	ng/ml		ng/ml	ng/ml	ng/ml	ng/ml	ng/ml
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	ng/ml	ng/ml	ng/ml	ng/ml	ng/ml																										
Assay Procedure:	<p>1. Bring all reagents and samples to room temperature (18 - 25 °C) before use. It is recommended that all standards and samples be run at least in duplicate. 2. Add 100 µL of each standard (see Reagent Preparation step 2) and sample into appropriate wells. Cover well and incubate for 2.5 hours at room temperature or over night at 4 °C with gentle shaking. 3. Discard 4. Add 100 µL of 1x prepared biotinylated antibody (Reagent Preparation step 6) to each well. Incubate for 1 hour at room temperature with gentle shaking. 5. Discard the solution. Repeat the wash as in step 3. 6. Add 100 µL of prepared Streptavidin solution (see Reagent</p>																														

## Application Details

Preparation step 7) to each well. Incubate for 45 minutes at room temperature with gentle shaking. 7. Discard the solution. Repeat the wash as in step 3. 8. Add 100 µL of TMB One-Step Substrate Reagent (Item H) to each well. Incubate for 30 minutes at room temperature in the dark with gentle shaking. 9. Add 50 µL of Stop Solution (Item I) to each well. Read at 450 nm immediately.

**Calculation of Results:** Calculate the mean absorbance for each set of duplicate standards, controls and samples, and subtract the average zero standard optical density. Plot the standard curve on log-log graph paper or using Sigma plot software, with standard concentration on the x-axis and absorbance on the y-axis. Draw the best-fit straight line through the standard points.

**Assay Precision:** Intra-Assay: CV<10%  
Inter-Assay: CV<12%

**Restrictions:** For Research Use only

## Handling

**Handling Advice:** Avoid repeated freeze-thaw cycles.

**Storage:** -20 °C

**Storage Comment:** The entire kit may be stored at -20°C for up to 1 year from the date of shipment. Avoid repeated freeze-thaw cycles. The kit may be stored at 4°C for up to 6 months. For extended storage, it is recommended to store at -80°C.

**Expiry Date:** 6 months

## Publications

**Product cited in:** Zong, Song, Wang, Xia, Hu, Han, Wang: "LFP-20, a porcine lactoferrin peptide, ameliorates LPS-induced inflammation via the MyD88/NF-κB and MyD88/MAPK signaling pathways." in: **Developmental and comparative immunology**, Vol. 52, Issue 2, pp. 123-31, (2015) ([PubMed](#)).

Zhang, Hou, Zhong, Li, Chen, Li, Wen, Wang, Liu, Zhong: "Porcine reproductive and respiratory syndrome virus activates inflammasomes of porcine alveolar macrophages via its small envelope protein E." in: **Virology**, Vol. 442, Issue 2, pp. 156-62, (2013) ([PubMed](#)).

ELISA

Image 1.

