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Human Angiogenin ELISA Kit

Catalog No: CK400 **Size:** 1 x 96 tests

Specificity:	Human Angiogenin
Sensitivity:	1.5 pg/ml
Range:	1.64 pg/ml to 50 ng/ml
Sample Type:	Cell supernatants, serum, plasma samples.

Introduction:

Angiogenesis is the preferred term for processes leading to the generation of new blood vessels through sprouting from already existing blood vessels and involves the migration and proliferation of endothelial cells from pre-existing vessels. Angiogenic factors are of clinical significance because they may be used to interfere directly with angiogenic processes involved, for example, in wound healing, inflammatory diseases, ischemic heart and peripheral vascular diseases, and myocardial infarctions.

The Human Angiogenin ELISA is an *in vitro* enzyme-linked immunosorbent assay for the quantitative measurement of Human Angiogenin in serum, plasma, and cell culture supernatants. This assay employs an antibody specific for Angiogenin coated on a 96-well plate. Standards and samples are pipetted into the wells and Angiogenin present in a sample is bound to the wells by the immobilized antibody. The wells are washed and Biotinylated Anti-Human Angiogenin antibody is added. After washing away unbound Biotinylated antibody, HRP-Streptavidin is pipetted to the wells. The wells are again washed, a TMB substrate solution is added to the wells and color develops in proportion to the amount of Angiogenin bound. The stop solution changes the color from blue to yellow, and the intensity of the color is measured at 450 nm.

Reagents and materials supplied in the kit:

Items	Quantity	Storage/Stability After Preparation
CK400-A Anti-Human Angiogenin Microplate: 12 strips x 8 wells	96 wells	1 month at 2-8°C
CK400-B Wash Buffer Concentrate (20x)	25 mL	1 month at 2-8°C
CK400-C Recombinant Human Angiogenin Standard 1 vial is enough to run each standard in duplicate.	2 vials	1 week at -80°C
CK400-D Assay Diluent A: Standard/Sample – Plasma/Serum	30 mL	n/a
CK400-E Assay Diluent B (5x): Standard/Sample - Cell Culture Medium	15 mL	1 month at 2-8°C
CK400-F Detection Antibody: Biotinylated Anti-Human Angiogenin Each vial is enough to coat ½ microplate.	2 vials	5 days at 2-8°C
CK400-G Streptavidin-HRP Concentrate (700x)	200 µl	Do not store and reuse.
CK400-H TMB One-Step Substrate Reagent (3, 3', 5, 5'-tetramethylbenzidine in buffered solution)	12 mL	n/a
CK400-I Stop Solution (0.2 M Sulfuric Acid)	8 mL	n/a

Storage of Kit Reagents:

Stable unopened for 6 months from date of shipment at 2-8°C or 1 year at -20°C. For extended stability, store at -80°C. Once opened, microplate strips and reagents are stable for 1 month at 2-8°C. Return unused strips to the pouch containing desiccant pack and reseal along the entire edge. **Avoid repeated freeze-thaw cycles**.

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Materials not provided:

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

Preparation of Kit Reagents:

1.Bring all reagents and samples to room temperature (18-25°C) before use.

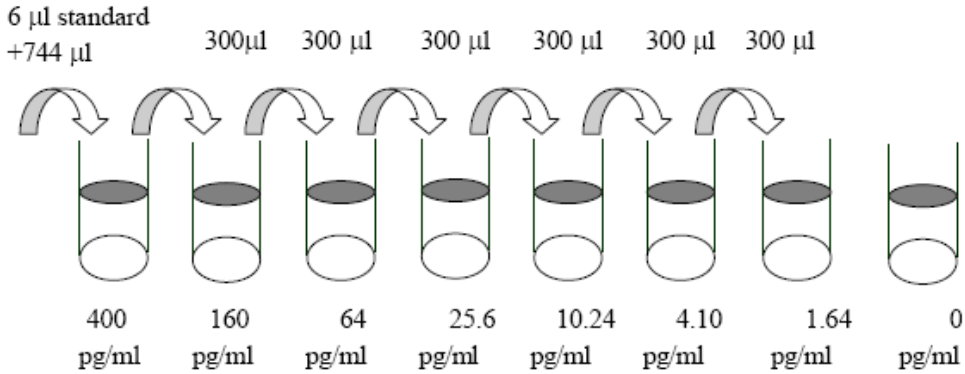
2. Assay Diluent B (**CK400-E**):
Dilute 5-fold with deionized or distilled water.

3. Sample Dilution:
If samples need to be diluted, Assay Diluent A (**CK400-D**) is used for dilution of serum/plasma samples and 1x Assay Diluent B is used for dilution of cell culture supernatants and urine. We recommend dilution of serum/plasma samples 1,000-10,000-fold.

Please note that levels of the target protein may vary in specimens. Optimal dilution factors for each sample must be determined by the investigator.

4. **Angiogenin Standard**:
Briefly centrifuge the vial of **CK400-C** (Recombinant Human Angiogenin Standard).
Add 400 µl Assay Diluent A (for serum/plasma samples) or 1x Assay Diluent B (for cell culture medium and urine) to prepare a 50 ng/mL standard.
Dissolve the powder thoroughly by gentle mixing.
Add 6 µl standard from the vial of Item C, into a tube with 744 µl Assay Diluent A or 1x Assay Diluent B to prepare a 400 pg/mL stock standard solution.
Add 450 µl Assay Diluent A or 1x Assay Diluent B into each tube. Use the stock standard solution to produce a dilution series (shown below in Figure 1).
Gently vortex to mix each tube thoroughly before the next transfer.
Assay Diluent A or 1x Assay Diluent B serves as the zero standard (0 pg/mL).

Figure 1



5. **Wash Buffer**:
If the Wash Concentrate (**CK400-B**) contains visible crystals, warm to RT and mix gently until dissolved.
Dilute 20 mL of Wash Buffer Concentrate into distilled water to yield 400 mL of 1x Wash Buffer.

6. **Detection Antibody:**
Briefly centrifuge Detection Antibody vial (**CK400-F**) before use.
Add 100 µl of 1x Assay Diluent B into the vial to prepare a detection antibody concentrate.
Mix gently (the concentrate can be stored at 2-4°C for 5 days).
The detection antibody concentrate should be diluted 80-fold with 1x Assay Diluent B and used in Step 4 of the ELISA Method.

7. **Streptavidin-HRP:**
Briefly spin Streptavidin-HRP Concentrate vial (**CK400-G**) and mix gently before use.
Streptavidin-HRP concentrate should be diluted 700-fold with 1x Assay Diluent B.

For example: Briefly centrifuge the vial (CK400-G) mix gently. Add 20 µl of Streptavidin-HRP concentrate into a tube with 14 ml 1x Assay Diluent B to prepare a 700-fold diluted Streptavidin-HRP solution.

ASSAY PROCEDURE:

Be sure to read **'Preparation of Kit Reagents'** before carrying out the assay.

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 **Preparation of Kit Reagents: Angiogenin Standard**) and sample into appropriate wells. Cover well and incubate for 2.5 hours at room temperature or overnight at 2-4°C with gentle shaking.

3. Discard the solution and wash 4 times with 1x Wash Solution (300 µl each). Wash by filling each well using a multi-channel Pipette or auto-washer. 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.

4. Add 100 µl of 1x prepared biotinylated antibody (see **Preparation of Kit Reagents: Detection Antibody**) to each well. Incubate for 1 hour at room temperature with gentle shaking.

5. Discard the solution and repeat the wash as in step 3.

6. Add 100 µl of prepared Streptavidin solution (see **Preparation of Kit Reagents: Streptavidin-HRP**) to each well. Incubate for 45 minutes at room temperature with gentle shaking.

7. Discard the solution and repeat the wash as in step 3.

8. Add 100 µl of TMB One-Step Substrate Reagent (**CK400-H**) to each well. Incubate for 30 minutes at room temperature in the dark with gentle shaking.

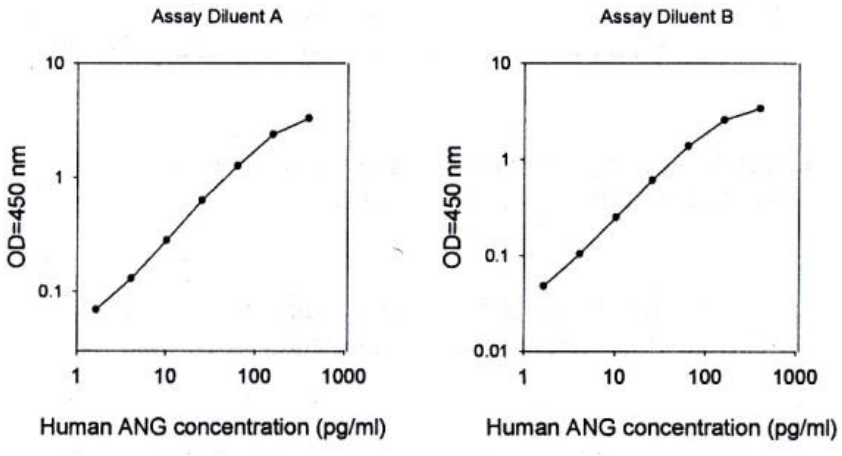
9. Add 50 µl of Stop Solution (**CK400-I**) to each well. Read at 450 nm immediately.

Assay Procedure Summary:

1. Prepare all reagents, samples and standards as instructed.
2. Add 100 µl standard or sample to each well. Incubate 2.5 hours at RT.
3. Add 100 µl prepared biotin antibody to each well. Incubate 1 hour at RT.
4. Add 100 µl prepared Streptavidin solution. Incubate 45 minutes at RT.
5. Add 100 µl TMB to each well. Incubate 30 minutes at RT.
6. Add 50 µl Stop Solution 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.

Typical Data:
Standard curve is for demonstration **ONLY**. A standard curve **MUST** be run with each assay.



Performance and Characteristics:
Sensitivity:
The minimum detectable dose of Angiogenin is typically less than 1.5 pg/mL.

Recovery:
Recovery was determined by spiking various levels of Human Angiogenin into Human serum, plasma and cell culture media. Mean recoveries are as follows:

Sample Type	Average % Recovery	Range (%)
Serum	95.95	82-105
Plasma	93.49	83-104
Cell culture media	95.37	85-104

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Linearity:

Sample Type		Serum	Plasma	Cell culture media
1:2	Average % of Expected	97	95	98
	Range (%)	83-104	85-103	85-105
1:4	Average % of Expected	96	95	95
	Range (%)	84-103	86-104	84-102

Reproducibility:
Intra-Assay: CV<10%
Inter-Assay: CV<12%

Specificity:

Cross Reactivity: This ELISA kit shows no cross-reactivity with any of the cytokines tested (e.g., human BDNF, BLC, ENA-78, FGF-4, IL-1 alpha, IL-1 beta, IL-2, IL-3, IL-4, IL-5, IL-7, IL-8, IL-9, IL-11, IL-12 p70, IL-12 p40, IL-13, IL-15, IL-309, IP-10, G-CSF, GM-CSF, IFN gamma, Leptin (OB), MCP-1, MCP-2, MCP-3, MDC, MIP-1 alpha, MIP-1 beta, MIP-1 delta, PARC, PDGF, RANTES, SCF, TARC, TGF-beta, TIMP-1, TIMP-2, TNF-alpha, TNF-beta, TPO, VEGF).

Troubleshooting Guide:

Problem	Cause	Solution
1. Standard curve	1. Inaccurate pipetting	1. Check pipettes
	2. Improper standard dilution	2. Ensure a brief spin of Item C and dissolve the powder thoroughly by a gentle mix.
2. Low signal	1. Too brief incubation times	1. Ensure sufficient incubation time; change Step 2 to overnight.
	2. Inadequate reagent volumes or improper dilution	2. Check pipettes and ensure correct preparation.
3. Large CV	1. Inaccurate pipetting	1. Check pipettes.
4. High background	1. Plate is insufficiently washed	1. Review the manual for proper wash. If using a plate washer, check that all ports are unobstructed.
	2. Contaminated wash buffer	2. Make fresh wash buffer.
5. Low sensitivity	1. Improper storage	1. Store your standard at <-20°C after reconstitution, others at 2-4°C. Keep substrate solution protected from light.
	2. Stop solution	2. Stop solution should be added to each well before measure.

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