Version: Datasheet-SE50061-A.0

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Speedy™ Human Angiogenin One-Step ELISA Kit Datasheet

Please read it entirely before use

Catalogue Number: SE50061

Size: **96T**

Sensitivity: 0.5 pg/mL

Range: 3.13-200 pg/mL

Usage: For the quantitative detection of human Angiogenin concentrations in serum, plasma, cell culture supernatant and cell lysate.

This product is for research use only and not for use in human or animal therapeutic or diagnostic.

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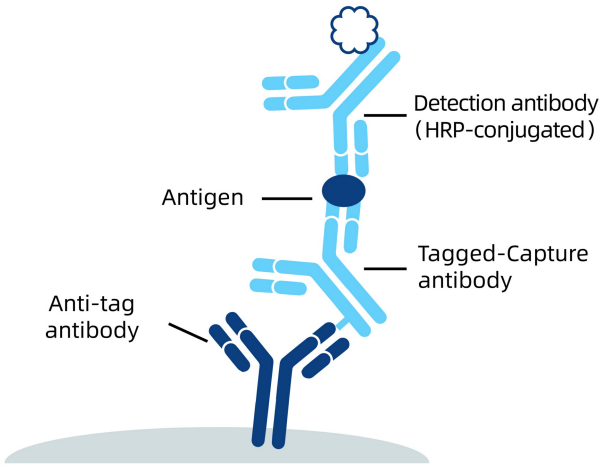
1. Background

Angiogenin (ANG), an angiogenic ribonuclease, is a member of the vertebrate-specific, secreted RNASE superfamily. Angiogenin, originally identified as a tumor angiogenic factor, was related with the growth and metastasis of numerous tumors. Angiogenin has been proposed as a permissive factor for angiogenesis induced by other angiogenic factors, including vascular endothelial growth factor (VEGF), basic fibroblast growth factor, acidic fibroblast growth factor, and epidermal growth factor. Angiogenin production and secretion may be stimulated by hypoxia. Increased angiogenin serum levels have been associated with the incidence and severity of several human tumors, including HCC.

2. Principle



An anti-tag antibody is pre-coated onto the bottom of wells. After adding antigen or samples, Tagged-Capture antibody and HRP-conjugated detection antibody, a sandwich complex is formed in the solution. TMB acts as a HRP substrate, and the solution color will change from colorless to blue. A stop solution containing sulfuric acid turns the solution yellow. The color intensity is proportional to the quantity of bound protein, which is measurable at 450 nm with the correction wavelength set at 630 nm.

3. Required Materials

3.1 A microplate reader capable of measuring absorbance at 450 nm with the correction wavelength set at 630 nm.

3.2 Calibrated, adjustable precision pipettes and disposable plastic tips. A manifold multi-channel pipette is recommended for large assays.

3.3 Plate washer: automated or manual.

3.4 Absorbent paper towels.

3.5 Glass or plastic tubes to prepare standard and sample dilutions.

3.6 Beakers and graduated cylinders.

3.7 Log-log or semi-log graph paper or computer and software for ELISA data analysis. A four-parameter logistic (4-PL) curve-fit is recommended. Proteintech data analysis website, https: //www.ptgcn.com/products/elisa-kits/.

3.8 Microplate thermostatic shaker.

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4. Kit Components and Storage

Microplate - 96 well microplate precoated an anti-tag antibody (8 well × 12 strips)	1 plate	**Unopened Kit:** Store at 2-8°C for 6 months or -20°C for 12 months. **Opened Kit:** All reagents stored at 2-8°C for 7 days. **Please use a new standard for each assay.**
Protein standard - 400 pg/bottle; lyophilized	2 bottles	
Capture antibody (100×) - 60 uL/vial*	1 vial	
Detection antibody, HRP-Conjugated (100×) - 60 uL/vial*	1 vial	
Sample Diluent PT 4B1 - 30 mL/bottle	1 bottle	
Detection Diluent - 15 mL/bottle	1 bottle	
Wash Buffer Concentrate (20×) - 30 mL/bottle	1 bottle	
Extraction Reagent - 15 mL/bottle	1 bottle	
Tetramethylbenzidine Substrate (TMB) - 12 mL/bottle	1 bottle	
Stop Solution - 12 mL/bottle	1 bottle	
Plate Cover Seals	4 pieces	

* Centrifugation immediately before use

5. Safety Notes

5.1 Avoid any skin and eye contact with Stop Solution and TMB. In case of contact, wash thoroughly with water.

5.2 Do not use the kit after the expiration date.

5.3 Do not mix or substitute reagents or materials from other kit lots or other sources.

5.4 Be sure to wear protective equipment such as gloves, masks and goggles during the experiment.

5.5 When using an automated plate washer, adding a 30 second soak period following the addition of Wash Buffer to improve assay precision

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6. Sample Collection and Storage

6.1 Serum: Allow blood samples to clot for 30 minutes, followed by centrifugation for 15 minutes at 1000xg. Clear serum can be assayed immediately or aliquoted and stored at -20°C. Avoid repeated freeze-thaw cycles.

6.2 Plasma: Use EDTA, heparin, or citrate as an anticoagulant for plasma collection. Centrifuge for 15 minutes at 1000xg within 30 minutes of collection. The plasma can be assayed immediately or aliquoted and stored at -20°C. Avoid repeated freeze-thaw cycles.

6.3 Cell Culture Supernatant: Remove particulates by centrifugation for 5 minutes at 500xg and assay immediately or aliquot and store samples at ≤ -20°C. Avoid repeated freeze-thaw cycles.

6.4 Cell Lysate:

1) Collect cells and wash by centrifuging at 500 x g for 5 minutes before resuspension in pre-cooled PBS buffer. Perform this step three times.

2) Count cells and then discard the supernatant.

3) Add protease inhibitor cocktail to the Extraction Reagent to a final concentration immediately prior to performing cell lysis.

4) Add 1 mL of Extraction reagent (containing protease inhibitor cocktail) Per 1 x 10^7 cells, Incubate cell suspension on ice for 30 minutes, use ultrasound to treat the samples.

5) Centrifuge cell lysate at 10,000 x g for 5 minutes at 4°C.

6) Measure the concentration of total protein in cell lysate using BCA assay. Where possible, keep samples on ice to avoid protein degradation.

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7. Reagent Preparation

7.1 Wash Buffer (1X): If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Add 30 mL of Wash Buffer Concentrate(20X) to 570 mL deionized or distilled water to prepare 1X Wash Buffer.

7.2 Antibody Cocktail (1X): Dilute 100X capture antibody and 100X HRP-conjugated detection antibody using Detection Diluent prior to assay. Suggested 1:100 dilution: 50 uL 100X capture antibody + 50 uL 100X Detection Antibody, HRP-conjugated + 4,900 uL Detection Diluent. Mix gently but thoroughly.

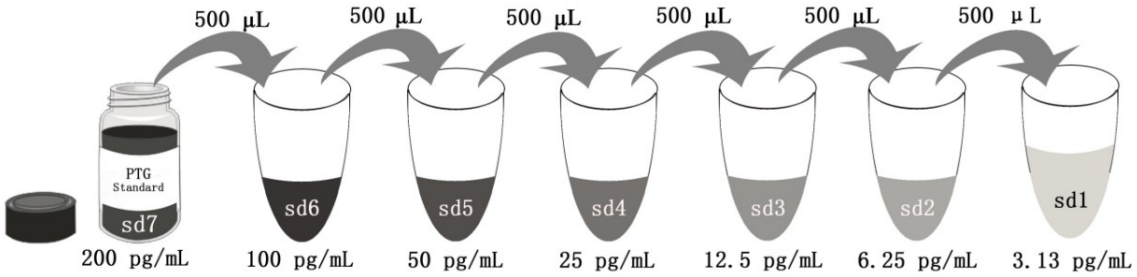
7.3 Sample Dilution: Different samples should be diluted with corresponding Sample Diluent. The minimum required dilution factor was 1:2; consequently, no more than 25 uL of undiluted sample was added per well.

Samples may require further dilution if the readout values are higher than the highest standard OD reading. Variations in sample collection, processing and storage may affect the results of the measurement.

Recommended Dilution for different sample types: 1:2,000 or 1:4,000 is recommended for human serum and plasma; 1:2 to 1:80 is recommended for cell culture supernatant; 1:20 or 1:40 is recommended for cell lysate.

7.4 Standard Serial Dilution:

Add 2 mL Sample Diluent PT 4B1 in protein standard.



Add # μL of Standard diluted in the previous step	—	500 μL	500 μL	500 μL	500 μL	500 μL	500 μL
# μL of Sample Diluent PT 4B1	**2000 μL**	500 μL	500 μL	500 μL	500 μL	500 μL	500 μL
	"sd7"	"sd6"	"sd5"	"sd4"	"sd3"	"sd2"	"sd1"

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8. Assay Procedure Summary

Bring all reagents to room temperature before use (Detection antibody, HRP-conjugated antibody can be used immediately). 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.

8.1 Preset the layout of the microplate, including control group, standard group and sample group, take out the required number of microplate strips and return excess strips to the foil pouch containing the drying reagent pack and reseal; store at 4°C immediately. Microplate strips should be used in one week.

8.2 Add 50 μL standard or sample to appropriate wells. To avoid high background always add samples or standards to the well before the addition of antibody cocktail.

8.3 Add 50 μL 1× Antibody Cocktail solution (refer to Reagent Preparation 7.2) to each well. Seal plate with cover seal and incubate at 37°C on a microplate thermostatic shaker set at 400 rpm for 1 hour (incubate at 37°C for 2 hours is recommended if thermostatic shaker is not available) .

8.4 Wash

1) Gently remove the cover seal. Discard the liquid from wells by aspirating or decanting. Remove any residual solution by tapping the plate a few times on fresh paper towels.

2) Wash 4 times with 1× Wash Buffer, using at least 350-400 μL per well. Following the last wash, firmly tap plates on fresh towels 10 times to remove residual Wash Buffer. Avoid getting any towel fibers in the wells or wells drying out completely.

8.5 Add 100 μL TMB substrate solution to each well, protected from light. Incubate at 37°C on a microplate thermostatic shaker set at 400 rpm for 15 to 20 minutes. (Substrate Solution should remain colorless until added to the plate.)

8.6 Add 100 μL Stop Solution to each well in the same order as addition of the TMB substrate. Note: Avoid skin and eye contact with the Stop solution.

8.7 Read results immediately on a microplate reader at a wavelength of 450 nm. If possible, perform a double wavelength readout (450 nm and 630 nm).

8.8 Data analysis: Calculate the average of the duplicate readings (OD value) for each standard and sample, and subtract the average of the zero standard absorbance. Construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis, four-parameter logistic curve- fit (4-PL) analysis is recommend. If the samples have been diluted, the fitting result must be multiplied by the dilution factor used.

Procedure summary

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Add 50 µL standard or sample to appropriate wells

Add 50 µL antibody cocktail solution (1x) to each well

Incubate at 37 °C on a microplate thermostatic shaker set at 400 rpm for 1 hour

Aspirate and wash each well four times with 400µL 1 x Wash Buffer

Add 100 µL TMB substrate solution to each well

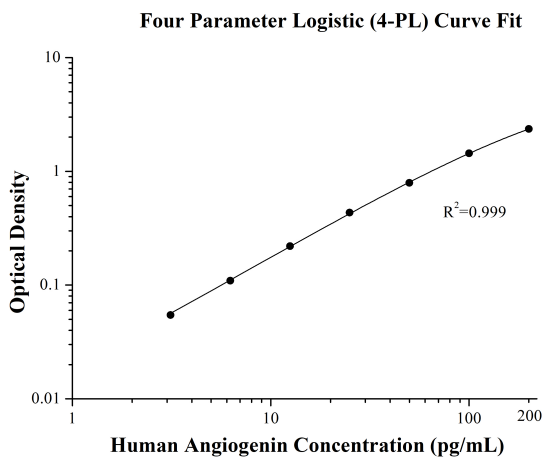
Incubate at 37 °C on a microplate thermostatic shaker set at 400 rpm for 15minutes

Add 100 µL Stop Solution to each well and Read OD immediately

9. Validation Data

9.1 Standard curve

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



(pg/mL)	O.D	Average	Corrected
0	0.0184 0.0192	0.0188	-
3.13	0.0733 0.0734	0.07335	0.05455
6.25	0.1291 0.1271	0.1281	0.1093
12.5	0.239 0.2384	0.2387	0.2199
25	0.4496 0.4549	0.45225	0.43345
50	0.8143 0.8044	0.80935	0.79055
100	1.4816 1.4403	1.46095	1.44215
200	2.3691 2.3763	2.3727	2.3539

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9.2 Precision

Intra-assay Precision (Precision within an assay) Three samples of known concentration were tested 8 times on one plate to assess intra-assay precision.

Inter-assay Precision (Precision between assays) Three samples of known concentration were tested in 16 separate assays to assess inter-assay precision.

Intra-assay Precision				
Sample	n	Mean (pg/mL)	SD	CV%
1	8	100.9	2.9	2.9
2	8	26.1	0.4	1.5
3	8	7.4	0.2	2.7

Inter-assay Precision				
Sample	n	Mean (pg/mL)	SD	CV%
1	16	100.2	2.4	2.4
2	16	25.6	0.7	2.7
3	16	7.2	0.3	4.2

9.3 Recovery

The recovery of human Angiogenin spiked to three different levels throughout the range of the assay in various matrices was evaluated.

Sample Type		Average% of Expected	Range (%)
Human plasma	1:8,000	90	77-97
	1:16,000	94	89-96
Cell culture supernatant	1:8	107	104-111
	1:16	108	105-111
Cell lysate	1:80	79	72-83
	1:160	82	75-86

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9.4 Sample values

Human plasma - human plasma samples were evaluated for the presence of human Angiogenin in this assay.

Sample Type	Mean (**ng/mL**)	Range (**ng/mL**)
Human plasma (n=16)	383.6	219.8-580.1

Cell culture supernatant - human peripheral blood mononuclear cells (1 x 10^6 cells/mL) were cultured in DMEM supplemented 10% fetal bovine serum, 50 µM β-mercaptoethanol, 2 mM L-glutamine, 100 U/mL penicillin and 100 µg/mL streptomycin sulfate. Cells were stimulated with 10 µg/mL PHA and 100 ng/mL recombinant human IL-2 for 1 day, 3 days and 5 days. Aliquots of the cell culture supernates were removed and assayed for levels of human Angiogenin.

Stimulated Condition	Day 1 (pg/mL)	Day 3 (pg/mL)	Day 5 (pg/mL)
	29.7	73.3	146.4

HepG2 were cultured in DMEM supplemented with 10% fetal bovine serum, 2.5 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 Angiogenin, and measured 4,792.7 pg/mL.

HT-29 were cultured in DMEM supplemented with 10% fetal bovine serum, 2.5 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 Angiogenin, and measured 520.9 pg/mL.

Cell lysate

	Human Angiogenin (pg/mL)	Total protein (**mg/mL**)
HepG2 cell lysate	1,949.1	1.1

9.5 Sensitivity

The minimum detectable dose of human Angiogenin is 0.5 pg/mL. This was determined by adding two standard deviations to the concentration corresponding to the mean O.D. of 20 zero standard replicates.

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9.6 Linearity

To assess the linearity of the assay, samples were diluted with the appropriate **Sample Diluent** to produce samples with values within the dynamic range of the assay.

(The human plasma was initially diluted 1:1,000. The cell culture supernatant was initially diluted 1:40. The cell lysate was initially diluted 1:10.)

		Human plasma	Cell culture supernatant	Cell lysate
1:2	Average% of Expected	100	100	100
	Range (%)	-	-	-
1:4	Average% of Expected	100	97	107
	Range (%)	96-104	96-98	105-108
1:8	Average% of Expected	99	99	120
	Range (%)	98-100	98-101	118-123
1:16	Average% of Expected	96	91	127
	Range (%)	96-97	86-96	126-128

9.7 Specificity

This assay recognizes natural and recombinant human Angiogenin.

The following factors prepared at 50 ng/mL were assayed and exhibited no cross-reactivity or interference.

Recombinant human:	Recombinant mouse:
EGF	GM-CSF
G-CSF	IL-1β
GM-CSF	
IL-1α	
IL-1β	
TGF-β1	

10. References

1. Miyake, M et al. Oncogene vol. 34,7 (2015): 890-901.
2. Hartmann, A et al. Cancer research vol. 59,7 (1999): 1578-83.
3. Kishimoto, Koji et al. Oncogene vol. 24,3 (2005): 445-56.
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