

## Human Angiostatin K1-3Fast ELISA Kit

Catalog Number: EA102929

### Assay Principle

The OriGene Human PLG Pre-Coated ELISA (Enzyme-Linked Immunosorbent Assay) kit is a solid phase immunoassay specially designed to measure Human PLG with a 96-well strip plate that is pre-coated with antibody specific for PLG. The detection antibody is a biotinylated antibody specific for PLG. The capture antibody is monoclonal antibody from mouse, the detection antibody is polyclonal antibody from goat. The kit contains recombinant Human PLG with immunogen: Expression system for standard: NSO, V98-S355(K1-3). The kit is analytically validated with ready to use reagents.

To measure Human PLG, add standards and samples to the wells, then add the biotinylated detection antibody. Wash the wells with PBS or TBS buffer, and add Avidin-Biotin-Peroxidase Complex (ABC-HRP). Wash away the unbound ABC-HRP with PBS or TBS buffer and add TMB. TMB is substrate to HRP and will be catalyzed to produce a blue color product, which changes into yellow after adding acidic stop solution. The density of the yellow product is linearly proportional to Human PLG in the sample. Read the density of the yellow product in each well using a plate reader, and benchmark the sample wells' readings against the standard curve to determine the concentration of Human PLG in the sample.

### Overview

<b>Product Name</b>	Human Angiostatin K1-3 Fast ELISA Kit
<b>Reactive Species</b>	Human
<b>Size</b>	96wells/kit, with removable strips.
<b>Description</b>	The Fast version of ELISA kits, assay takes less than 1.5 hours. Detect Human Plasminogen/PLG with < 10pg/ml sensitivity. Format: 96-well plate with removable strips. Compatible samples: serum, plasma and cell culture supernates. This is a TMB colorimetric sandwich ELISA kit with short assay time and fast experiment set up. Plasminogen/PLG tissue specificity: Present in plasma and many other extracellular fluids. It is synthesized in the liver.
<b>Sensitivity</b>	< 10pg/ml *The sensitivity or the minimum detectable dose (MDD) is the lower limit of target protein that can be detected by the kit. It is determined by adding two standard deviations to the mean O.D. value of twenty (20) blank wells and calculating the corresponding concentration.
<b>Detection Range</b>	156pg/ml-10000pg/ml
<b>Storage Instructions</b>	Store at 4 °C for 6 months, at -20 °C for 12 months. Avoid multiple freeze-thaw cycles (Shipped with wet ice.)
<b>Uniprot ID</b>	P00747

## Technical Details

<b>Capture/Detection Antibodies</b>	<i>The capture antibody is monoclonal antibody from mouse, the detection antibody is polyclonal antibody from goat.</i>
<b>Specificity</b>	<i>Natural and recombinant Human PLG</i>
<b>Immunogen</b>	<i>Expression system for standard: NSO, V98-S355(K1-3)</i>
<b>Cross Reactivity</b>	<i>There is no detectable cross-reactivity with other relevant proteins.</i>

## Notice Before Application

Please read the following instructions before starting the experiment.

1. To inspect the validity of experiment operation and the appropriateness of sample dilution proportion, pilot experiment using standards and a small number of samples is recommended.
2. Before using the Kit, spin tubes and bring down all components to the bottom of tubes.
3. Don't let 96-well plate dry, for dry plate will inactivate active components on plate.
4. Don't reuse tips and tubes to avoid cross contamination.
5. Avoid using the reagents from different batches together.

## Kit Components/Materials Provided

<b>Description</b>	<b>Quantity</b>	<b>Volume</b>
<b>Anti-Human PLG Pre-coated 96-well strip microplate</b>	<b>1</b>	<b>12 strips of 8 wells</b>
<b>Human PLG Standard</b>	<b>2</b>	<b>100ng/tube</b>
<b>Human PLG Biotinylated antibody (50x)</b>	<b>1</b>	<b>130 µl</b>
<b>Avidin-Biotin-Peroxidase Complex (30x)</b>	<b>1</b>	<b>400 µl</b>
<b>Sample Diluent</b>	<b>1</b>	<b>30ml</b>
<b>Antibody Diluent</b>	<b>1</b>	<b>12ml</b>
<b>Avidin-Biotin-Peroxidase Diluent</b>	<b>1</b>	<b>12ml</b>
<b>Color Developing Reagent (TMB)</b>	<b>1</b>	<b>10ml</b>
<b>Stop Solution</b>	<b>1</b>	<b>10ml</b>

Plate Sealers

4

Piece

## Required Materials That Are Not Supplied

Microplate Reader capable of reading absorbance at 450nm.

Automated plate washer (optional)

Pipettes and pipette tips capable of precisely dispensing 0.5 µl through 1 ml volumes of aqueous solutions.

Multichannel pipettes are recommended for large amount of samples.

Deionized or distilled water.

500ml graduated cylinders.

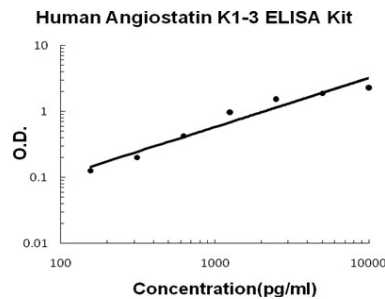
Test tubes for dilution.

## Human Angiostatin K1-3 Fast ELISA Kit (EA102929) Standard Curve Example

Highest O.D. value might be higher or lower than in the example. The experiment result is statistically significant if the highest O.D. value is no less than 1.0.

Concentration 0 (pg/ml)	156	312	625	125000	25000	5000	10000	
O.D.	0.024	0.127	0.201	0.426	0.968	1.538	1.868	2.294

Human Angiostatin K1-3 ELISA Kit standard curve



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

## Intra/Inter Assay Variability

OriGene spend great efforts in documenting lot to lot variability and make sure our assay kits produce robust data that are reproducible.

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

**Inter-Assay Precision (Precision across assays):** Three samples of known concentration were tested in separate assays to assess inter-assay precision.

Sample	Intra-Assay Precision			Inter-Assay Precision		
	1	2	3	1	2	3
n	16	16	16	24	24	24
Mean(pg/ml)	241	1987	5716	218	1908	5845
Standard deviation	12.05	79.48	337.24	13.73	91.58	432.53
CV(%)	5%	4%	5.9%	4.8%	4.8%	7.4%

## Reproducibility

To assay reproducibility, three samples with differing target protein concentrations were assayed using four different lots.

Lots	Lot1 (pg/ml)	Lot2 (pg/ml)	Lot3 (pg/ml)	Lot4 (pg/ml)	Mean (pg/ml)	Standard Deviation	CV (%)
Sample 1	241	249	248	219	239	12.09	5%
Sample 2	1987	1892	1804	1733	1854	95.22	5.1%
Sample 3	5716	5780	5230	5301	5506	243.6	4.4%

\*number of samples for each test n=16.

## Preparation Before The Experiment

Item	Preparation
All reagents	Bring all reagents to 37°C prior to use. Also the TMB incubation time estimate (20-25min) is based on 37°C.
Wash buffer	Dissolve the included powder in 1000ml of deionized water. Excess wash buffer can be stored for up to one week at 4°C.
Biotinylated Anti-Human PLG antibody	It is recommended to prepare this reagent immediately prior to use by diluting the Human ANGPT1 Biotinylated antibody (50x) 1:50 with Antibody Diluent. Prepare 50 µl by adding 1 µl of Biotinylated antibody (50x) to 49 µl of Antibody Diluent. Mix gently and thoroughly and use within 2 hours of generation.
Avidin-Biotin-Peroxidase Complex	It is recommended to prepare this reagent immediately prior to use by diluting the Avidin-Biotin-Peroxidase Complex (30x) 1:30 with Avidin-Biotin-Peroxidase Diluent. Prepare 300 µl by adding 10 µl of Avidin-Biotin-Peroxidase Complex (30x) to 290 µl of Avidin-Biotin-Peroxidase Diluent. Mix gently and thoroughly and use within 2 hours of generation.

Human PLG Standard	It is recommended that the standards be prepared no more than 2 hours prior to performing the experiment. Use one 100ng of lyophilized Human PLG standard for each experiment. Gently spin the vial prior to use. Reconstitute the standard to a stock concentration of 100ng/ml using 1ml of sample diluent. Allow the standard to sit for a minimum of 10 minutes with gentle agitation prior to making dilutions.
Microplate	The included microplate is coated with capture antibodies and ready-to-use. It does not require additional washing or blocking. The unused well strips should be sealed and stored in the original packaging.

## Dilution of Human PLG Standard

1. Number tubes 1-8. Final Concentrations to be Tube # 1 – 10000pg/ml, #2 – 5000pg/ml, #3 – 2500pg/ml, #4 – 1250pg/ml, #5 – 625pg/ml, #6 – 312.5pg/ml, #7 – 156.25pg/ml, #8 – 0.0 (Blank).
2. To generate standard #1, add 100µl of the reconstituted standard stock solution of 100ng/ml and 900µl of sample diluent to tube #1 for a final volume of 1000µl. Mix thoroughly.
3. Add 300 µl of sample diluent to tubes # 2-7.
4. To generate standard #2, add 300 µl of standard #1 from tube #1 to tube #2 for a final volume of 600 µl. Mix thoroughly.
5. To generate standard #3, add 300 µl of standard #2 from tube #2 to tube #3 for a final volume of 600 µl. Mix thoroughly.
6. Continue the serial dilution for tube #4-7.
7. Tube #8 is a blank standard to be used with every experiment.

## Sample Preparation and Storage

These sample collection instructions and storage conditions are intended as a general guideline and the sample stability has not been evaluated.

Sample Type	Procedure
Cell culture supernatants	Clear sample of particulates by centrifugation, assay immediately or store samples at -20°C.
Serum	Use a serum separator tube (SST) and allow serum to clot at room temperature for about four hours. Then, centrifuge for 15 min at approximately 1,000 x g. assay immediately or store samples at -20°C.
Plasma	Collect plasma using heparin or EDTA as an anticoagulant. Centrifuge for 15 min at approximately 1,000 x g. Assay immediately or store samples at -20°C.  *Note: it is important to not use anticoagulants other than the ones described above to treat plasma for other anticoagulants could block the antibody binding site.

## Sample Dilution

The target protein concentration should be estimated and appropriate sample dilutions should be selected such that the final protein concentration lies near the middle of the linear dynamic range of the assay.

*It is recommended to prepare 150  $\mu$ l of sample for each replicate to be assayed. The samples should be diluted with sample diluent and mixed gently.*

## **Assay protocol**

*It is recommended that all reagents and materials be equilibrated to 37°C/room temperature prior to the experiment (see Preparation Before The Experiment if you have missed this information).*

- 1. Prepare all reagents and working standards as directed previously.*
- 2. Remove excess microplate strips from the plate frame and seal and store them in the original packaging.*
- 3. Add 50  $\mu$ l of the standard, samples, or control per well. And add 50  $\mu$ l of the prepared 1x Biotinylated Anti-Human ANGPT1 antibody per well. Add 50  $\mu$ l of the sample diluent buffer and 50  $\mu$ l of the prepared 1x Biotinylated Anti-Human ANGPT1 antibody into the control well (Zero well). At least two replicates of each standard, sample, or control is recommended.*
- 4. Cover with the plate sealer provided and incubate for 60 minutes at RT.*
- 5. Wash the plate 3 times with the 1x wash buffer.*
  - a. Discard the liquid in the wells into an appropriate waste receptacle. Then, invert the plate on the benchtop onto a paper towel and tap the plate to gently blot any remaining liquid. It is recommended that the wells are not allowed to completely dry at any time.*
  - b. Add 300  $\mu$ l of the 1x wash buffer to each assay well. (For cleaner background incubate for 60 seconds between each wash).*
  - c. Repeat steps a-b 2 additional times.*
- 6. Add 100  $\mu$ l of the prepared 1x Avidin-Biotin-Peroxidase Complex into each well. Cover with plate sealer provided and incubate for 15 minutes at RT.*
- 7. Wash the plate 5 times with the 1x wash buffer.*
  - a. Discard the liquid in the wells into an appropriate waste receptacle. Then, invert the plate on the benchtop onto a paper towel and tap the plate to gently blot any remaining liquid. It is recommended that the wells are not allowed to completely dry at any time.*
  - b. Add 300  $\mu$ l of the 1x wash buffer to each assay well. (For cleaner background incubate for 60 seconds between each wash).*
  - c. Repeat steps a-b 4 additional times.*
- 8. Add 90  $\mu$ l of Color Developing Reagent to each well and incubate in the dark for 30 minutes at RT (or 25-30 minutes at 37°C). (The optimal incubation time must be empirically determined. A guideline to look for is blue shading the top four standard wells, while the remaining standards remain clear.)*
- 9. Add 100  $\mu$ l of Stop Solution to each well. The color should immediately change to yellow.*
- 10. Within 30 minutes of stopping the reaction, the O.D. absorbance should be read with a microplate reader at 450nm.*

## **Data Analysis**

*Average the duplicate readings for each standard, sample, and control. Subtract the average zero standard O.D. reading.*

*It is recommended that a standard curve be created using computer software to generate a four parameter logistic (4-PL) curve-fit. A free program capable of generating a four parameter logistic (4-PL) curve-fit can be found online at: [www.myassays.com/four-parameter-logistic-curve.assay](http://www.myassays.com/four-parameter-logistic-curve.assay).*

*Alternatively, plot the mean absorbance for each standard against the concentration. The measured concentration in the sample can be interpolated by using linear regression of each average relative OD against the standard curve generated using curve fitting software. This will generate an adequate but less precise fit of the data.*



**OriGene Technologies Inc.**

9620 Medical Center Drive, Suite 200, Rockville, MD 20850

Phone: 1.888.267.4436

Fax: 301-340-9254

Email: [techsupport@origene.com](mailto:techsupport@origene.com)

Web: [www.origene.com](http://www.origene.com)

---

*For diluted samples, the concentration reading from the standard curve must be multiplied by the dilution factor.*

## ***Background on PLG***

*Ang K1-3 is a single, non-glycosylated polypeptide chain containing 259 amino acids. It represents a proteolytic fragment of plasminogen containing the first three kringle structures. Ang K1-3 reduces endothelial cell proliferation and acts as a potent inhibitor of angiogenesis and tumor growth. It displays increased inhibitory activity ( $ED_{50} = 70$  nM) relative to kringles 1-4 ( $ED_{50} = 135$  nM).*