Human ANG ELISA Kit
(hANG-ELISA)

Cat. No. EK0305

96 Tests in 8 x 12 divisible strips

Background

Angiogenin (Ang), also known as ribonuclease 5, is a protein that in humans is encoded by the ANG gene. Angiogenin is a potent stimulator of new blood vessel formation. It hydrolyzes cellular tRNAs resulting in decreased protein synthesis, and it is similar to pancreatic ribonuclease. Hooper et al. (2003) reviewed the evidence that angiogenins are involved in host defense and noted that inflammation provokes upregulated ANG mRNA expression in liver and an increase in detectable ANG protein in serum. Weremowicz et al. (1989, 1990) assigned the human angiogenin gene to chromosome 14q11.

ScienCell's human ANG ELISA Kit is based on standard sandwich enzyme-linked immune-sorbent assay technology. Human ANG specific-specific polyclonal antibodies are precoated onto 8 x 12 divisible strips. The human specific detection polyclonal antibodies are biotinylated. The test samples and biotinylated detection antibodies are subsequently added to the wells and then washed with PBS or TBS buffer. Avidin-Biotin-Peroxidase Complex is added, and unbound conjugates are washed away with PBS or TBS buffer. HRP substrate TMB is used to visualize HRP enzymatic reaction. TMB is catalyzed by HRP to produce a blue color product that changes into yellow after adding acidic stop solution. The intensity of yellow is proportional to the amount of human ANG that is captured in the strips.

Size 96 Tests in 8 x 12 divisible strips

Assay type Sandwich ELISA

Range 78 pg/ml-5000 pg/ml

Sensitivity < 12 pg/ml

Specificity No detectable cross-reactivity with any other cytokine.

Storage Store at 4°C for frequent use, at -20°C for infrequent use.
Avoid multiple freeze-thaw cycles

Shipping Shipped on gel ice.

Expiration Four months at 4°C and eight months at -20°C.

Application For quantitative detection of human ANG in serum, plasma, body fluids, tissue lysates or cell culture supernatants.

Kit components

1. Lyophilized recombinant human ANG standard: 10ng/tube×2.
2. 8 x 12 divisible pre-coated with anti- human ANG antibody.
3. Sample diluent buffer: 30 ml
4. Biotinylated anti- human ANG antibody: 130µl, dilution 1:100.
5. Antibody diluent buffer: 12ml.
6. Avidin-Biotin-Peroxidase Complex (ABC): 130µl, dilution 1:100.
7. ABC diluent buffer: 12ml.
8. TMB color developing agent: 10ml.
9. TMB stop solution: 10ml.

Materials Required But Not Provided

1. Microplate reader.
2. Automated plate washer.
3. Adjustable pipettes and pipette tips. Multichannel pipettes are recommended for large number of samples.
4. Clean tubes and Eppendorf tubes.
5. Washing buffer (neutral PBS or TBS).

Preparation of 0.01M TBS: Add 1.2g Tris, 8.5g Nacl; 450µl of purified acetic acid or 700µl of concentrated hydrochloric acid to 1000ml H2O and adjust pH to 7.2-7.6. Finally, adjust the total volume to 1L.

Preparation of 0.01 M PBS: Add 8.5g sodium chloride, 1.4g Na2HPO4 and 0.2g NaH2PO4 to 1000ml distilled water and adjust pH to 7.2-7.6. Finally, adjust the total volume to 1L.

Usage

This product is for research use only. It is not approved for use in humans, animals, or *in vitro* diagnostic procedures.

Reference

1. Weremowicz S, Fox EA, Morton CC, Vallee BL (1990). "Localization of the human angiogenin gene to chromosome band 14q11, proximal to the T cell receptor alpha/delta locus". Am J Hum Genet 47 (6): 973–81.
2. "Entrez Gene: ANG angiogenin, ribonuclease, RNase A family, 5". http://www.ncbi.nlm.nih.gov/sites/entrez?Db=gene&Cmd=ShowDetailView&TermToSearch=283.

Protocol for Human ANG ELISA (96 well format)

Notes before you begin

1. To inspect the validity of experiment operation and the appropriateness of sample dilution proportion, a pilot experiment using standards and a small number of samples is recommended.
2. The TMB Color developing agent should be colorless and transparent before using.
3. Before using the kit, spin tubes and bring down all components to the bottom of tubes.
4. A duplicate well assay is recommended for both standard and samples.
5. Do not let wells dry, as this will inactivate active components in wells.
6. Do not reuse tips and tubes to avoid cross contamination.
7. Avoid using reagents from different batches.

8. In order to avoid marginal effect of plate incubation due to temperature difference (reaction may be stronger in the marginal wells), it is suggested that the diluted ABC and TMB solution be pre-warmed in 37°C for 30 minutes before use.

Preparation

Sample Preparation and Storage

Store samples to be assayed within 24 hours at 2-8°C. For long-term storage, aliquot and freeze samples at -20°C. Avoid repeated freeze-thaw cycles.

- **Cell culture supernatant, tissue lysate or body fluids**: Remove particulates by centrifugation, analyze immediately or aliquot and store at -20°C
- **Serum**: Allow the serum to clot in a serum separator tube (about 4 hours) at room temperature. Centrifuge at approximately 1000 X g for 15 minutes. Analyze the serum immediately or aliquot and store frozen at -20°C.
- **Plasma**: Collect plasma using heparin, EDTA, citrate as an anticoagulant. Centrifuge for 30 minutes at 1000 x g within 30 minutes of collection. Analyze immediately or aliquot and store frozen at -20°C. Citrate is not recommended as an anticoagulant.

Sample Dilution Guideline

The user needs to estimate the concentration of the target protein in the sample and select a proper dilution factor so that the diluted target protein concentration falls near the middle of the linear regime in the standard curve. Dilute the sample using the provided diluent buffer. The following is a guideline for sample dilution. Several trials may be necessary in practice. **The sample must be well mixed with the diluent buffer**.

- **High target protein concentration (50-500ng/ml)**. The working dilution is 1:100. i.e. Add 1 µl sample into 99 µl sample diluent buffer.
- **Medium target protein concentration (5-50 ng/ml)**. The working dilution is 1:10. i.e. Add 10 µl sample into 90 µl sample diluent buffer.
- **Low target protein concentration (78-5000 pg/ml)**. The working dilution is 1:2. i.e. Add 50 µl sample to 50 µl sample diluent buffer.
- **Very Low target protein concentration (≤78 pg/ml)**. No dilution necessary, or the working dilution is 1:2.

Reagent Preparation and Storage

A. Reconstitution of the human ANG standard: ANG standard solution should be prepared no more than 2 hours prior to the experiment. Two tubes of ANG standard (10ng per tube) are included in each kit. Use one tube for each experiment.

- 10,000 pg/ml of human ANG standard solution: Add 1 ml sample diluent buffer into one tube, keep the tube at room temperature for 10 minutes and mix thoroughly.
- 5000 pg/ml→78 pg/ml of human ANG standard solutions: Label 7 Eppendorf tubes with 5000 pg/ml, 2500 pg/ml, 1250 pg/ml, 625 pg/ml, 312 pg/ml, 156 pg/ml, 78 pg/ml respectively. Aliquot 0.3 ml of the sample diluent buffer into each tube. Add 0.3 ml of the above 10, 000pg/ml ANG standard solution into 1st tube and mix. Transfer 0.3 ml from 1st tube to 2nd tube and mix. Transfer 0.3 ml from 2nd tube to 3rd tube and mix, and so on.

Note: The standard solutions are best used within 2 hours. The 10ng/ml standard solution should be stored at 4°C for up to 12 hours, or at -20°C for up to 48 hours. Avoid repeated freeze-thaw cycles.

B. Preparation of biotinylated anti- human ANG antibody working solution: The solution should be prepared no more than 2 hours prior to the experiment.

- The total volume should be: 0.1ml/well x (the number of wells). (Allowing 0.1-0.2 ml more than total volume)

- Biotinylated anti-human ANG antibody should be diluted in 1:100 with the antibody diluent buffer and mixed thoroughly.

C. Preparation of Avidin-Biotin-Peroxidase Complex (ABC) working solution: The solution should be prepared no more than 1 hour prior to the experiment.

- The total volume should be: 0.1ml/well x (the number of wells). (Allowing 0.1-0.2 ml more than total volume)
- Avidin- Biotin-Peroxidase Complex (ABC) should be diluted in 1:100 with the ABC dilution buffer and mixed thoroughly.

Assay Procedure

The ABC working solution and TMB color developing agent must be kept warm at 37°C for 30 minutes before use. When diluting samples and reagents, they must be mixed completely and evenly. Standard ANG detection curve should be prepared for each experiment. The user will decide sample dilution fold by crude estimation of ANG amount in samples.

1. Aliquot 0.1ml per well of the 5000 pg/ml, 2500 pg/ml, 1250 pg/ml, 625 pg/ml, 313 pg/ml, 156 pg/ml, 78 pg/ml, human ANG standard solutions into the precoated strips. Add 0.1ml of the sample diluent buffer into the control well (**blank well**). Add 0.1ml of each properly diluted sample of human serum, plasma, body fluids, tissue lysates or cell culture supernatants to each empty well. See "**Sample Dilution Guideline**" above for details. We recommend that each human ANG standard solution and each sample is measured in duplicate.
2. Seal the strips with the cover and incubate at 37°C for 90 minutes.
3. Remove the cover, discard strips content, and blot the strips onto paper towels or other absorbent material. **Do NOT** let the wells completely dry at any time.
4. Add 0.1ml of biotinylated anti-human ANG antibody working solution into each well and incubate the strips at 37°C for 60 minutes.
5. Wash strips 3 times with 0.01M TBS or 0.01M PBS, and each time let washing buffer stay in the wells for 1 minutes. Discard the washing buffer and blot the strips onto paper towels or other absorbent material. (**Strips Washing Method**: Discard the solution in the strips without touching the side walls. Blot the strips onto paper towels or other absorbent material. Soak each well with at least 0.3 ml PBS or TBS buffer for 1~2 minutes. Repeat this process two additional times for a total of THREE washes. Note: For automated washing, aspirate all wells and wash THREE times with PBS or TBS buffer, overfilling wells with PBS or TBS buffer. Blot the strips onto paper towels or other absorbent material).
6. Add 0.1ml of prepared ABC working solution into each well and incubate the strips at 37°C for 30 minutes.
7. Wash strips 5 times with 0.01M TBS or 0.01M PBS, and each time let washing buffer stay in the wells for 1-2 minutes. Discard the washing buffer and blot the strips onto paper towels or other absorbent material.(See Step 5 for strips washing method).
8. Add 90 μl of prepared TMB color developing agent into each well and incubate strips at 37°C in dark for 25-30 minutes (**Note**: For reference only, the optimal incubation time should be determined by end user. And the shades of blue can be seen in the wells with the four most concentrated human ANG standard solutions; the other wells show no obvious color).
9. Add 0.1ml of prepared TMB stop solution into each well. The color changes into yellow immediately.
10. Read the O.D. absorbance at 450 nm in a microplate reader within 30 minutes after adding the stop solution.

For calculation, (the relative O.D.450) = (the O.D.450 of each well) – (the O.D.450 of blank well). The standard curve can be plotted as the relative O.D.450 of each standard solution (Y) vs. the respective concentration of the standard solution (X). The human ANG concentration of the samples can be interpolated from the standard curve.

Note: if the samples measured were diluted, multiply the dilution factor to the concentrations from interpolation to obtain the concentration before dilution.

Summary

1. Add samples and standards and incubate the strips at 37°C for 90 minutes. Do not wash.
2. Add biotinylated antibodies and incubate the strips at 37°C for 60 minutes. Wash strips 3 times with 0.01M TBS.
3. Add ABC working solution and incubate the strips at 37°C for 30 minutes. Wash strips 5 times with 0.01M TBS.
4. Add TMB color developing agent and incubate the strips at 37°C in dark for 25-30 minutes.
5. Add TMB stop solution and read.

Typical Data Obtained from Human ANG
(TMB reaction incubate at 25°C for 30 min)

Concentration (pg/ml)	0.0	78	156	313	625	1250	2500	5000
Absorbance (450 nm)	0.038	0.400	0.708	0.986	1.457	1.907	2.251	2.342

Typical Human ANG ELISA Kit Standard Curve

This standard curve was generated for demonstration purpose only. A standard curve must be run with each assay.