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# Bovine Amphiregulin (AREG) ELISA Kit

**User's Manual**

**Catalogue Number: SL0412Bo**

**48 or 96 Tests**

**For Research Use Only**

**Store all reagents between 2-8℃**

**Validity Period: Six (6) months**

**For use with serum, plasma, culture media, or any biological fluids.**

**Read the Materials Safety Data Sheet (MSDS) and follow all handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Materials Safety Data Sheets (MSDS) are available at https://www.sunlongbiotech.com/article.php?id=1762**

**For Research Use Only.**

**Not for Therapeutic or Diagnostic Applications.**

**Please follow the kit instructions to perform the procedure.**

# Bovine Amphiregulin (AREG)ELISA Kit

***FOR RESEARCH USE ONLY***

## Product Information

## Intended Use

Enzyme immunoassay for the quantitative detection of Bovine AREG antigen in serum, plasma, culture media, or any biological fluid.

## Principle

The quantitative detection of Bovine AREG antigen is based on the ELISA (Enzyme-Linked Immunosorbent Assay) technique. The microplate wells are pre-coated with an antibody specific to Bovine AREG. After incubation, the corresponding antigen in the sample or standards will bind to the immobilized antibody. After washing the microplate wells to remove all unbound sample material, horseradish peroxidase (HRP) labeled antibody conjugate is added, which binds to the antigen, forming antibody-antigen-enzyme-labeled antibody complex upon incubation. In a second wash step, all unbound material is removed. The immune complex formed by the bound conjugate is visualized by adding Tetramethylbenzidine (TMB) substrate, which gives a blue color reaction in wells containing the complex. A stop solution containing acid terminates the reaction, producing a yellow end-point coloration proportional to the amount of specific antigen present in the sample. Absorbance is measured at 450 nm using an ELISA microwell plate reader. Analysis is completed by comparing the OD values of samples to the standard curve.

## Reagent and Materials provided with the kit

| | Materials provided with the kit | 96T | 48T | Storage |
|---|---|---|---|---|
| 1 | Instructions for Use | 1 | 1 | R.T. |
| 2 | Adhesive films/membrane | 2 | 2 | R.T. |
| 3 | Sealed bags | 1 | 1 | R.T. |
| 4 | Microplate | 1 | 1 | 2-8℃ |
| 5 | Standard: 270pg/ml | 0.5ml×1 bottle | 0.5ml×1 bottle | 2-8℃ |
| 6 | Standard diluent | 1.5ml×1 bottle | 1.5ml×1 bottle | 2-8℃ |

| | | | | |
|---|---|---|---|---|
| 7 | HRP-Conjugate Reagent | 6ml×1 bottle | 3ml×1 bottle | 2-8℃ |
| 8 | Sample Diluent | 6ml×1 bottle | 3ml×1 bottle | 2-8℃ |
| 9 | Chromogen Solution A | 6ml×1 bottle | 3ml×1 bottle | 2-8℃ |
| 10 | Chromogen Solution B | 6ml×1 bottle | 3ml×1 bottle | 2-8℃ |
| 11 | Stop Solution | 6ml×1 bottle | 3ml×1 bottle | 2-8℃ |
| 12 | Wash Solution Concentrate | 20ml (30X*) ×1bottle | 20ml (20X*) ×1bottle | 2-8℃ |

*X denotes the product is concentrated 30 or 20 times for 96 and 48 tests, respectively

For potential hazardous substances please check the safety data sheet.

The kit allows the performance of 96 or 48 reactions, including blank and standard wells.

# Materials required but not provided

1. ELISA Microtiter plate reader, equipped for the measurement of absorbance at 450 nm
2. Incubator at 37℃
3. Manual or automatic equipment for rinsing Microtiter plates
4. Pipettes to deliver volumes between 10 and 1000µl
5. Vortex tube mixer
6. Distilled/Deionized water
7. Graduated measuring cylinder
8. Wash bottle
9. Paper towels/absorbent paper
10. Timer
11. Disposable tubes

# Storage

Store the kit at 2-8°C. The opened reagents are stable up to the expiry date stated on the kit when stored at 2-8°C. Return the unused plate strips to the aluminum foil bag immediately, squeeze out air and seal tightly. Keep the desiccant inside and store at 2-8℃ refrigerated. Use up within 1 month, max 3 months. Discard directly if mold, condensation or uneven liquid adhesion on well walls is found.

# Sample preparation

1. **Serum**

Collect whole blood into a non-anticoagulated (plain, red-top) tube. Allow the blood to clot undisturbed at room temperature for 10–20 minutes. Then centrifuge at 2,000–3,000 rpm for 20 minutes to separate the serum by removing the clot. If precipitates form during storage, centrifuge the sample again before use

2. **Plasma**

Collect whole blood into tubes containing anticoagulants such as EDTA or citrate. After incubating at room temperature for 10–20 minutes, centrifuge at 2,000–3,000 rpm for 20

minutes. Carefully collect the plasma supernatant. If precipitates form during storage, centrifuge the sample again before use.

3. **Urine**

Collect urine in sterile tubes. Centrifuge at 2,000–3,000 rpm for 20 minutes and carefully collect the clear supernatant. If precipitates appear during storage, centrifuge the sample again. Prepare **cerebrospinal and pleuroperitoneal fluids** following the same procedure as for urine.

4. **Cell samples**

Collect culture supernatant aseptically and centrifuge at 2,000–3,000 rpm for 20 minutes. Carefully collect the clarified supernatant. To detect intracellular components, dilute cells to $1\times10^6$/mL with Phosphate Buffered Saline (PBS) pH 7.2–7.4 and lyse by repeated freezing and thawing. Centrifuge the lysate under the same conditions and collect the supernatant. If precipitates appear during storage, centrifuge again before use.

5. **Tissue samples**

Tissue samples are cut, weighed, frozen in liquid nitrogen, and stored at -80℃ until use. Homogenize the tissue in PBS (pH 7.4) on ice or at 4℃. Centrifuge at 2,000–3,000 rpm for 20 minutes and carefully collect the supernatant. Aliquot the supernatant for ELISA and future analysis.

Note that for processing methods for uncommon ELISA samples, visit https://www.sunlongbiotech.com/article.php?id=1735

**Notes:**

1. Perform sample preparation and ELISA assay promptly after collection. Extract samples following relevant protocols. If immediate testing is not possible, store samples at -20°C. Avoid repeated freeze-thaw cycles to preserve sample integrity

2. Our kits are not compatible with samples containing sodium Azide ($NaN_3$), as it inhibits the activity of horseradish peroxidase (HRP), compromising assay performance.

3. It is very important to bring all reagents and samples to room temperature and mix them before starting the procedure

## Preparation of reagents

Wash Solution Concentrate should be brought to room temperature and should be diluted before starting the test procedure. If crystals have formed in the Wash Solution Concentrate, warm at 30 – 40℃ until they have completely dissolved.

**Wash Solution (1X)**

1. Pour the entire contents (20 mL) of the Wash Solution Concentrate (20X for 48 tests or 30X for 96 tests) into a clean 1,000 mL graduated cylinder. Bring to final volume to 400mL for 48 test or 600 mL for 96 tests with distilled or deionized water.

2. Mix gently to avoid foaming.

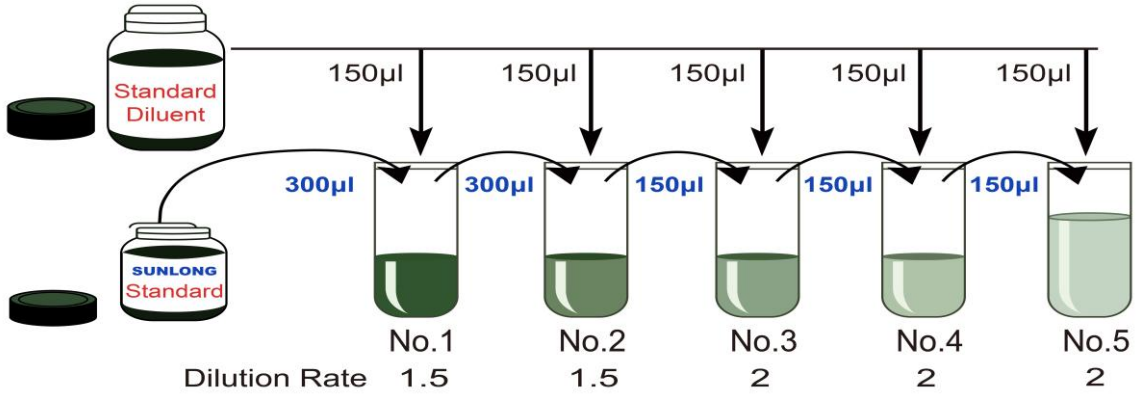
3. Transfer to a clean wash bottle and store at 2–25°C.

Please note that Wash Solution (1X) is stable for 30 days.

**Standard Dilution**

Prepare 5 tubes containing 150µl each in small tubes first with the Standard Diluent as shown below. Mix each tube thoroughly before the next transfer.

| Concentrations | Description | Dilutions |
|---|---|---|
| 270pg/ml | Stock | |
| 180pg/ml | Standard No.1 | 300µl Stock + 150µl Standard diluents |
| 120pg/ml | Standard No.2 | 300µl Standard No.1 + 150µl Standard diluents |
| 60pg/ml | Standard No.3 | 150µl Standard No.2 + 150µl Standard diluent |
| 30pg/ml | Standard No.4 | 150µl Standard No.3 + 150µl Standard diluent |
| 15pg/ml | Standard No.5 | 150µl Standard No.4 + 150µl Standard diluent |



| 270pg/ml | 180pg/ml | 120pg/ml | 60pg/ml | 30pg/ml | 15pg/ml |
|---|---|---|---|---|---|

Pipette the 50ul from each tube to two microplate wells, with a total of ten wells of duplicated standards.

## Procedure

1. **Preparation of Microplate.** Label the microplate wells sequentially with the corresponding sample numbers. Include ten wells for the five duplicated standards and one empty well as a blank control (well A1). Do not introduce samples or HRP Conjugate reagent into the blank well (well A1); however, continue to perform all subsequent steps as per the standard protocol. Remove any unused microplate strips from the holder and store them sealed in the provided foil bag with desiccant at 2–8°C.
2. **Pipetting the standards and samples.** Add 50µl of reconstituted standards to their respective wells. For sample wells, add 40µl of sample diluent followed by 10µl of sample. Carefully dispense samples to the bottom of the wells without touching the walls. Mix gently by shaking to ensure proper mixing.
3. **Incubation.** Incubate the Microplate 30 minutes at 37°C after sealing with an Adhesive film/membrane.
4. **Plate Washing.** Wash the strips five (5) times with 400µl of 1X Wash Solution per well. Soak strips for 30 seconds in between washes. When using an automated plate washer, be sure to recalibrate it so that the tips do not touch the bottom of the wells. Also, take caution when manually dispensing and aspirating into and out of the wells/ Remove residual Wash Buffer by tapping the plate upside down on paper towels without letting the wells dry out.

Note: Washing is important! Insufficient washing results in poor precision and false results.

5. **Conjugate Addition.** Add 50µl HRP-Conjugate reagent to each well except the blank control well (well A1).
6. **Conjugate Incubation.** Incubate the Microplate 30 minutes at 37°C after sealing with an Adhesive film/membrane as in Step 3.
7. **Plate Washing.** Wash the strips five (5) times with 400µl of 1X Wash Solution per well. Soak strips for 30 seconds in between washes. Remove residual Wash Buffer by tapping the plate upside down on paper towels without letting the wells dry out, as in Step 5. Do not

touch the bottom of the wells either during manual or automated washing.

Note: Washing is important! Insufficient washing results in poor precision and false results.

8. **Substrate Addition and Incubation.** Add 50µl Chromogen Solution A and 50µl Chromogen Solution B to each well, mix with gentle shaking, and incubate at 37℃ for 15 minutes. This should be done while avoiding a light source.
9. **Stop Reaction**. Add 50µl of Stop Solution to each well to terminate the enzymatic reaction. Upon addition, the color will change from blue to yellow, indicating the reaction has been successfully stopped
10. **Measurement.** Remove any drops of water and fingerprints on the bottom of the plate and confirm there are no bubbles on the surface of the liquid. Measure the optical density (O.D.) at 450 nm using a microtiter plate reader. Set the O.D. value of the blank control well to zero. Complete all readings within 15 minutes after adding the stop solution to ensure accurate results.

Note that due to technical reasons, if the ELISA Microtiter plate reader cannot be adjusted to zero using the Blank, subtract the Blank absorbance value from all other absorbance values measured to obtain reliable results

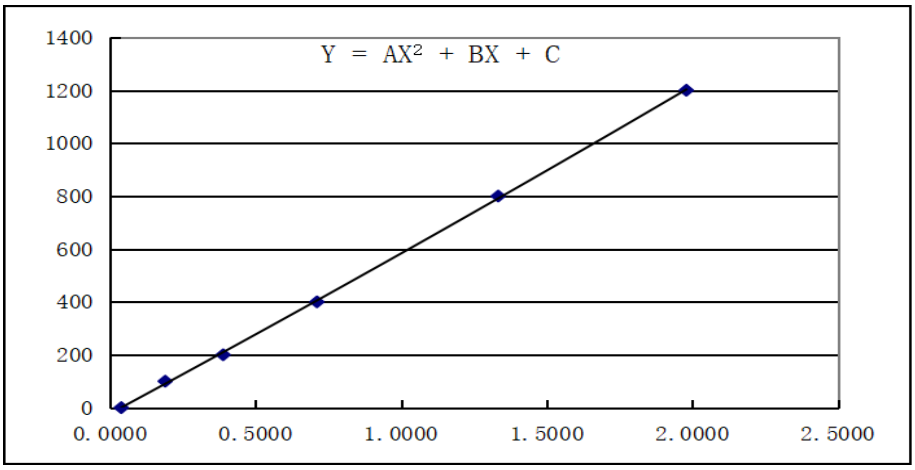
# Evaluation of the result

1. Calculate the average absorbance values for each set of duplicate standards and samples. Duplicates should be within 20% of the mean value.

2. Create a standard curve by plotting the mean absorbance for each Bovine AREG concentration. Draw a best fit curve through the points of the graph (a 5-parameter curve fit is recommended)

3. To determine the concentration of the sample, a horizontal line is extended from this mean absorbance value to intersect the standard curve. The point of intersection corresponds to the concentration of the analyte in the sample by extrapolation or interpolation from the standard curve.

**Standard Curve Analysis (Second-Order Polynomial Regression)**




**This diagram is for reference only，Specific calculation methods and steps refer to:**

**https://www.sunlongbiotech.com/article.php?id=36**

# Precision

Intra-assay Precision (Precision within an assay): 3 samples with low, middle, and high-level Bovine AREG were tested 20 times on one plate, respectively.

Inter-assay Precision (Precision between assays): 3 samples with low, middle, and high-level Bovine AREG were tested on 3 different plates, 8 replicates in each plate.

CV(%) = SD/mean X 100

Intra-Assay: CV<10%

Inter-Assay: CV<12%

# Assay range

3.1pg/ml-200pg/ml

# Sensitivity

0.6pg/ml

## Precautions

1. Store the kit at 2-8°C upon receipt. Before use, bring the kit to room temperature. Remove any unused strips from the Bovine AREG Antibody-coated plate, reseal them in the provided zip-lock foil bag with desiccant, and keep at 2-8°C.

2. Precipitates may form in the concentrated wash buffer. Warm the buffer to dissolve precipitates; this will not affect assay performance.

3. Utilize an accurate pipette to minimize experimental errors. Samples must be added to the microplate within five minutes. When processing a large quantity of samples, employing a multichannel pipette is advised for efficiency.

4. Include a standard curve in every assay, with replicate wells recommended to enhance reliability. If a sample's optical density (OD) exceeds that of the highest standard, the sample should be diluted (by a factor of n) before reassessment. When calculating the original AREG concentration, multiply by the total dilution factor (n × 5).

5. To prevent cross-contamination, use adhesive film/membrane only once.

6. Protect the substrate from exposure to light to maintain its stability and reactivity.

7. All procedures must strictly follow the manufacturer's instructions. Results should be obtained using a microtiter plate reader according to protocol specifications.

8. Treat all samples, wash buffer, and waste as potentially infectious.

9. Do not mix reagent from different kit lots.

## Storage and Validity

1. Storage: 2-8℃.
2. Valid Duration: 6 months

# Troubleshooting

| Possible Case | Solution |
|---|---|
| **Poor Standard Curve** | |
| • Improper standard curve preparation | • Ensure accurate operation of the dilution |
| • Incomplete washing and aspiration | • Adequate washing and adequate aspiration |
| • Inaccurate Pipetting | • Check and Calibrate pipettes |

| | |
|---|---|
| **High Background** | |
| • Improper washing | • Increase the duration of soaking before aspiration |
| • Contaminated Substrate | • Replace Substrate. Use a clean substrate and avoid cross-contamination by replacing the Adhesive film/membrane |
| • Non-specific binding of the antibody | • Replace with another purified antibody or blocking buffer |
| • The plate is not sealed completely | Follow the instructions strictly |
| • Incorrect incubation temperature | • Incubate at 37℃ |
| • Substrate exposed to light before use | • Keep the substrate in a dark place |
| • Contaminated wash buffer | • Use a clean buffer and a sterile filter |

| **Weak Signal** | |
|---|---|
| • Improper washing | • Increase the duration of soaking before aspiration |
| • Incorrect incubation temperature | • Incubate at 37°C |
| • Not enough antibody were used | • Increase the concentration of the antibody |
| • Reagents are contaminated | • Use new reagents |
| • Pipettes are not clean | • A clean pipette should be used |

| **No Signal** | |
|---|---|
| • Reagents are contaminated | • Use new reagents |
| • Sample prepared incorrectly | • Make sure samples are diluted accordingly |
| • Not enough antibody were used | • Increase the concentration of antibody |
| • Wash buffer contains sodium azide | • Use a new wash buffer without sodium azide |
| • HRP was not added | • Add HRP according to the instructions |
| **Poor Precision** | |
| • Imprecise/ inaccurate pipetting | • Check and calibrate pipettes |
| • Incomplete washing of the wells | • Ensure wells are thoroughly washed by filling each well with wash buffer and completely removing all residual antibody solutions before proceeding to the next wash step |