AREG Chemi-Luminescent ELISA Kit (Human)
(OKCD03311)
Lot# KD5156
Instructions for use

For the quantitative measurement of AREG in serum, plasma, tissue homogenates, cell lysates, cell culture supernatant and other biological fluids.

Lot to lot variations can occur. Refer to the manual provided with the kit.

This product is intended for research use only.

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1. Background

Principle

Aviva Systems Biology AREG Chemi-Luminescent ELISA Kit (Human) (OKCD03311) is based on standard sandwich enzyme-linked immuno-sorbent assay technology. An antibody specific for AREG has been pre-coated onto a 96-wellplate (12 x 8 Well Strips). Standards or test samples are added to the wells, incubated and removed. A biotinylated detector antibody specific for AREG is added, incubated and followed by washing. Avidin-Peroxidase Conjugate is then added, incubated and unbound conjugate is washed away. An enzymatic reaction is produced through the addition of a luminol substrate which is catalyzed by the HRP to produce light emission. The light emission is read by a luminometer (or photo-multiplier equipped instrument) and the intensity of the emitted light is proportional to the amount of sample AREG captured in well.

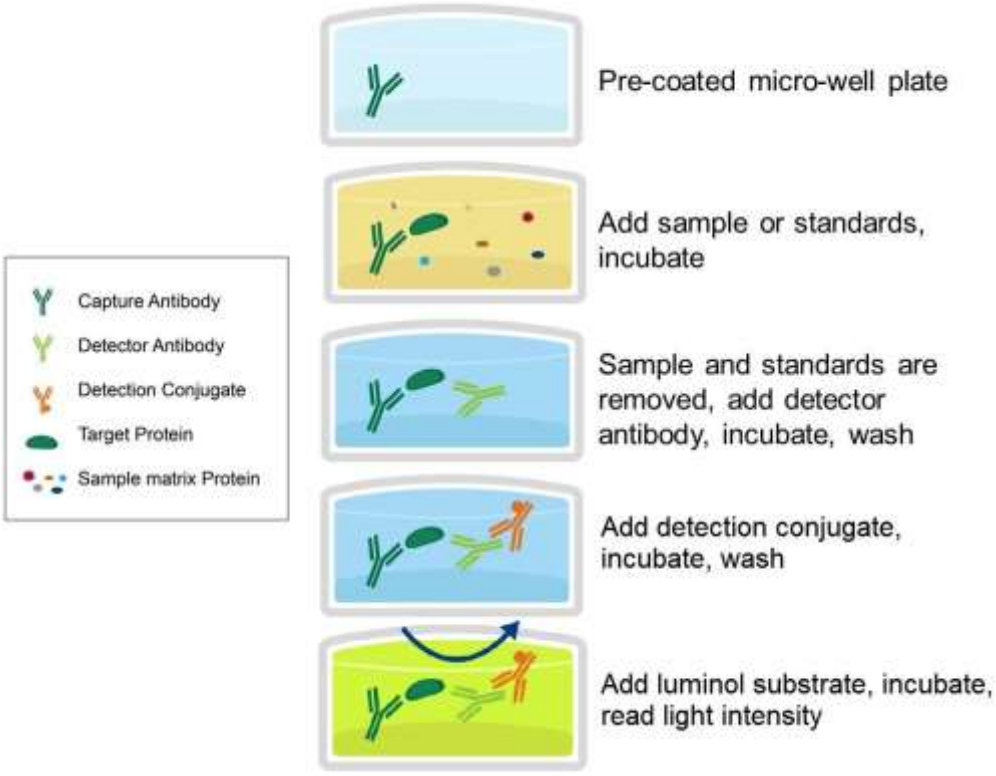
Background

Ligand of the EGF receptor/EGFR. Autocrine growth factor as well as a mitogen for a broad range of target cells including astrocytes, Schwann cells and fibroblasts.

General Specifications

General Specifications	
Range	1.37 - 1,000 pg/mL
LOD	< 0.59 pg/mL (Derived by linear regression of OD_{450} of the Mean Blank + 2xSD)
Specificity	Human Amphiregulin UniProt ID: P15514 GeneID: 374 Target Alias: AR, AREGB, CRDGF, MGC13647, SDGF
Cross-Reactivity	No detectable cross-reactivity with other relevant proteins

2. Assay Summary



3. Storage and Stability

• Open kit immediately upon receipt. Store components at -20°C (NOTE: exceptions below) for 6 months or until expiration date. Avoid any freeze/thaw cycles.

4. Kit Components

•The following reagents are the provided contents of the kit.

Description	Quantity	Storage Conditions
Anti-AREG Microplate	96 Wells (12 x 8 Well strips)	-20°C for 6 months
AREG Lyophilized Standard	2 x 1 ng	
100X Biotinylated AREG Detector Antibody	1 x 120 µL	
100X Avidin-HRP Conjugate	1 x 120 µL	
Standard Diluent	1 x 20 mL	4°C for 6 months
Detector Antibody Diluent	1 x 12 mL	
Conjugate Diluent	1 x 12 mL	
30X Wash Buffer	1 x 20 mL	
100X Luminol Substrate	1 x 2 mL	
Substrate Diluent	1 x 10 mL	

5. Precautions

• Read instructions fully prior to beginning use of the assay kit.

• Any deviations or modifications from the described method or use of other reagents could result in a reduction of performance.

• Reduce exposure to potentially harmful substances by wearing personal protective lab equipment including lab coats, gloves and glasses.

• For information on hazardous substances included in the kit please refer to the Material Safety Data Sheet (MSDS).

• Kit cannot be used beyond the expiration date on the label.

6. Required Materials Not Supplied

• Luminometer or photo-multiplier tube (PMT) equipped microplate reader capable of the following parameters: lag time 30.0 seconds, read time 1.0 seconds per well.

• Automated plate washer (optional).

• Pipettes capable of precisely dispensing 0.5 µL through 1 mL volumes of aqueous solutions.

• Pipettes or volumetric glassware capable of precisely measuring 1 mL through 100 mL of aqueous solutions.

• New, clean tubes and/or micro-centrifuge tubes for the preparation of standards or samples.

• Absorbent paper or paper toweling.

• Distilled or deionized ultrapure water.

• 37°C Incubator (optional)

7. Technical Application Tips

• Do not mix or substitute components from other kits.

• To ensure the validity of experimental operation, it is recommended that pilot experiments using standards and a small selection of sample dilutions to ensure optimal dilution range for quantitation.

• Samples exhibiting light intensity measurements higher than the highest standard should be diluted further in the appropriate sample dilution buffers.

• Prior to using the kit, briefly spin component tubes to collect all reagents at the bottom.

• Replicate wells are recommended for standards and samples.

• Cover microplate while incubating to prevent evaporation.

• Do not allow the microplate wells dry at any point during the assay procedure.

• Do not reuse tips or tube to prevent cross contamination.

• Avoid causing bubbles or foaming when pipetting, mixing or reconstituting.

• Completely remove of all liquids when washing to prevent cross contamination.

• Prepare reagents immediately prior to use and do not store, with the exception of the top standard.

• Equilibrate all materials to ambient room temperature prior to use (standards exception).

• For optimal results for inter- and intra-assay consistency, equilibrate all materials to room temperature prior to performing assay (standards exception) and perform all incubations at 37°C.

• Pipetting less than 1 µL is not recommended for optimal assay accuracy.

• Once the procedure has been started, all steps should be completed without interruption. Ensure that all reagents, materials and devices are ready at the appropriate time.

• Incubation times will affect results. All wells should be handled in the same sequential order and time intervals for optimal results.

• Samples containing precipitates, fibrin strands or bilirubin, or are hemolytic or lipemic might cause inaccurate results due to interfering factors.

• Luminol Substrate is easily contaminated and labile. Handle carefully and protect from light.

8. Reagent Preparation

• Equilibrate all materials to room temperature prior to use and use immediately.

8.1 Human AREG Assay Standards

8.1.1 Prepare the standards no greater than 2 hours prior to performing experiment. Standards should be held on ice until use in the experiment.

8.1.2 Reconstitute one vial of the provided 500 pg **Lyophilized Standard** for each experiment. Prepare a stock **1,000 pg/mL Standard** by reconstituting one tube of **Lyophilized Standard** as follows:

8.1.2.1 Gently spin or tap the vial at 6,000 – 10,000 rpm for 30 seconds to collect all material at the bottom.

8.1.2.2 Add 0.5 mL of **Standard Diluent** to the vial.

8.1.2.3 Seal the vial then mix gently and thoroughly.

8.1.2.4 Leave the vial at ambient temperature for 15 minutes.

8.1.3 Prepare a set of serially diluted standards as follows:

8.1.3.1 Label tubes with numbers 2 – 8.

8.1.3.2 Use the reconstituted **1,000 pg/mL Standard** from step 8.1.2 as the high standard point (Tube #1).

8.1.3.3 Add 600 µL of **Standard Diluent** to Tube #'s 2 – 8.

8.1.3.4 Prepare **Standard #2** by adding 300 µL of **1,000 pg/mL Standard** (Tube #1) to Tube #2. Mix gently and thoroughly.

8.1.3.5 Prepare **Standard #3** by adding 300 µL of **Standard #2** from Tube #2 to Tube #3. Mix gently and thoroughly.

8.1.3.6 Prepare further serial dilutions through Tube #7. Reference the table below as a guide for serial dilution scheme.

8.1.3.7 Tube #8 is a blank standard (only **Standard Diluent**), which should be included with every experiment.

Standard Number (Tube)	Standard To Dilute	Volume Standard to Dilute (µL)	Volume Standard Diluent (µL)	Total Volume (µL)	Final Concentration
1	500 pg/mL Reconstituted Standard	NA	500	500	1,000 pg/mL
2	1,000 pg/mL	300	600	900	333.33 pg/mL
3	333.33 pg/mL	300	300	600	111.11 pg/mL
4	111.11 pg/mL	300	300	600	37.04 pg/mL
5	37.04 pg/mL	300	300	600	12.35 pg/mL
6	12.35 pg/mL	300	300	600	4.12 pg/mL
7	4.12 pg/mL	300	300	600	1.37 pg/mL
8	NA	0	300	300	0.0 (Blank)

8.2 1X Biotinylated AREG Detector Antibody

8.2.1 Prepare the **1X Biotinylated AREG Detector Antibody** immediately prior to use by diluting the **100X Biotinylated AREG Detector Antibody** 1:100 with **Detector Antibody Diluent**.

8.2.2 For each well strip to be used in the experiment (8-wells) prepare 1,000 µL by adding 10 µL of **100X Biotinylated AREG Detector Antibody** to 990 µL **Detector Antibody Diluent**.

8.2.3 Mix thoroughly and gently. Hold no longer than 2 hours prior to using in procedure. Do not store at 1X concentration for future use.

8.3 1X HRP-Avidin Conjugate

8.3.1 Prepare the **1X Avidin-HRP Conjugate** immediately prior to use by diluting the **100X Avidin-HRP Conjugate** 1:100 with **Conjugate Diluent**.

8.3.2 For each well strip to be used in the experiment (8-wells) prepare 1,000 µL by adding 10 µL of **100X Avidin-HRP Conjugate** to 990 µL **Conjugate Diluent**.

8.3.3 Mix thoroughly and gently. Hold no longer than 2 hours prior to using in procedure. Do not store at 1X concentration for future use.

8.4 1X Luminol Substrate

8.4.1 Prepare the **1X Luminol Substrate** immediately prior to use by diluting the **100X Luminol Substrate** 1:100 with **Substrate Diluent**.

8.4.2 For each well strip to be used in the experiment (8-wells) prepare 1,000 µL by adding 10 µL of **100X Luminol Substrate** to 990 µL **Substrate Diluent**.

8.4.3 Mix thoroughly and gently. Hold no longer than 2 hours prior to using in procedure. Do not store at 1X concentration for future use.

8.5 1X Wash Buffer

8.5.1 If crystals have formed in the 30X **Wash Buffer** concentrate, equilibrate to room temperature and mix gently until crystals have completely dissolved.

8.5.2 Add the entire 20 mL contents of the 30X **Wash Buffer** bottle to 580 mL of ultra-pure water to a clean > 1,000 mL bottle or other vessel.

8.5.3 Seal and mix gently by inversion. Avoid foaming or bubbles.

8.5.4 Store the **1X Wash Buffer** at room temperature until ready to use in the procedure. Store the prepared **1X Wash Buffer** at 4°C for no longer than 1 week. Do not freeze.

8.6 Microplate Preparation

- Micro-plates are provided ready to use and do not require rinsing or blocking.
- Unused well strips should be returned to the original packaging, sealed and stored at 4°C.
- Equilibrate microplates to ambient temperatures prior to opening to reduce potential condensation.

9. Sample Preparation

9.1 Sample Preparation and Storage

• Store samples to be assayed at 2-8°C for 24 hours prior being assayed.

• For long term storage, aliquot and freeze samples at -20°C. Avoid repeated freeze-thaw cycles.

• Samples not indicated in the manual must be tested to determine if the kit is valid.

• Prepare samples as follows:

• **Serum** - Use a serum separator tube (SST) and allow samples to clot for two hours at room temperature or overnight at 4°C before centrifugation for 20 minutes at 1,000 x g. Remove serum and assay immediately or aliquot and store samples at -20°C or -80°C. Avoid repeated freeze-thaw cycles.

• **Plasma** - Collect plasma using EDTA, or heparin as an anticoagulant. Centrifuge for 15 minutes at 1,000 x g at 2-8°C within 30 minutes of collection. Assay immediately or aliquot and store samples at -20°C or -80°C. Avoid repeated freeze-thaw cycles.

• **Tissue Homogenates** – Rinse 100 mg of tissue with 1X PBS, then homogenize in 1 mL of 1X PBS and store overnight at -20°C. Perform two freeze-thaw cycles to break the cell membranes, then centrifuge the homogenates for 5 minutes at 5,000 x g, 2-8°C. Remove the supernatant and assay immediately. Alternatively, aliquot and store samples at -20°C or -80°C. Centrifuge the sample again after thawing before the assay. Avoid repeated freeze-thaw cycles.

• **Cell Lysates** –

Adherent Cells - Remove media and rinse cells once with ice-cold PBS (pH 7.2-7.4). Scrape cells off the plate and transfer to an appropriate tube. Dilute cell suspension with 1x PBS (pH 7.2-7.4) for a final cell concentration of 100 million/mL then store overnight at -20°C. Perform two freeze-thaw cycles to break up the cell membranes, then centrifuge the cell lysates for 5 minutes at 5,000 x g, 2 - 8°C. Collect the supernatant. Cell lysates should be assayed immediately or aliquoted and stored at -20°C. Centrifuge the sample again after thawing before the assay. Avoid repeated freeze-thaw cycles.

Suspended Cells - Collect cells and centrifuge for 5 minutes at 1,000 x g, 2 - 8°C. Remove the supernatant and resuspend cells with 1x PBS (pH 7.2-7.4). Centrifuge for 5 minutes at 1,000 x g, 2 - 8°C. Remove the supernatant. Dilute cell with 1x PBS (pH 7.2-7.4), for a final cell concentration of 100 million/mL. Store overnight at -20°C. Perform two freeze-thaw cycles to break up the cell membranes, then centrifuge the cell lysates for 5 minutes at 5,000 x g, 2 - 8°C. Collect the supernatant. Cell lysates should be assayed immediately or aliquotted and stored at -20°C. Centrifuge the sample again after thawing before the assay. Avoid repeated freeze-thaw cycles.

• **Cell culture supernatant and other biological fluids** - Centrifuge samples for 20 minutes at 1,000×g. Remove particulates and assay immediately or store samples in aliquot at -20°C or -80°C for later use. Avoid repeated freeze/thaw cycles.

10. Assay Procedure

• Equilibrate all reagents and materials to ambient room temperature prior to use in the procedure.

• Optimal results for intra- and inter-assay reproducibility will be obtained when performing incubation steps at 37°C as indicated below.

10.1 Determine the required number of wells and return any remaining unused wells and desiccant to the pouch.

10.2 Add 100 µL of serially titrated standards, diluted samples or blank into wells of the **Anti-AREG Microplate**. At least two replicates of each standard, sample or blank is recommended.

10.3 Cover the plate with the plate sealer and incubate at 37°C for 60 minutes.

10.4 Remove the plate sealer and discard the liquid in the wells by rigorously flicking into an acceptable waste receptacle or aspiration.

10.5 Gently blot any remaining liquid from the wells by tapping inverted on the benchtop onto paper toweling. Do not allow the wells to completely dry at any time.

10.6 Add 100 µL of prepared **1X Biotinylated AREG Detector Antibody** to each well.

10.7 Cover with the plate sealer and incubate at 37°C for 60 minutes.

10.8 Discard the liquid in the wells by rigorously flicking into an acceptable waste receptacle or aspiration.

10.9 Gently blot any remaining liquid from the wells by tapping inverted on the benchtop onto paper toweling. Do not allow the wells to completely dry at any time.

10.10 Wash plate 3 times with **1X Wash Buffer** as follows:

10.10.1 Add 300 µL of **1X Wash Buffer** to each assay well.

10.10.2 Incubate for 1-2 minutes.

10.10.3 Discard the liquid in the wells by rigorously flicking into an acceptable waste receptacle.

10.10.4 Gently blot any remaining liquid from the wells by tapping inverted on the benchtop onto paper toweling. Do not allow the wells to completely dry at any time.

10.10.5 Repeat steps 10.10.1 through 10.10.4 **two** more times.

10.11 Add 100 µL of prepared **1XAvidin-HRP Conjugate** into each well, cover with plate sealer and incubate at 37°C for 30 minutes.

10.12 Discard the liquid in the wells by rigorously flicking into an acceptable waste receptacle or aspiration.

10.13 Gently blot any remaining liquid from the wells by tapping inverted on the benchtop onto paper toweling. Do not allow the wells to completely dry at any time.

10.14 Wash plate **5 times** with **1X Wash Buffer** as in Step 10.10.

10.15 Add 100 µL of **1X Luminol Substrate** to each well. Cover the plate with a plate seasler and incubate at 37°C for 10 minutes **in the dark**.

10.16 Read the RLU (relative light units) with a standard luminometer or photo-multiplier tube equipped instrument.

11. Calculation of Results

For analysis of the assay results, calculate the **Relative Light Units (RLU)** for each test or standard well as follows

(Relative Light Units) = (Mean Sample Well Light Unit Emission) – (Mean Blank Well Light Unit Emission)

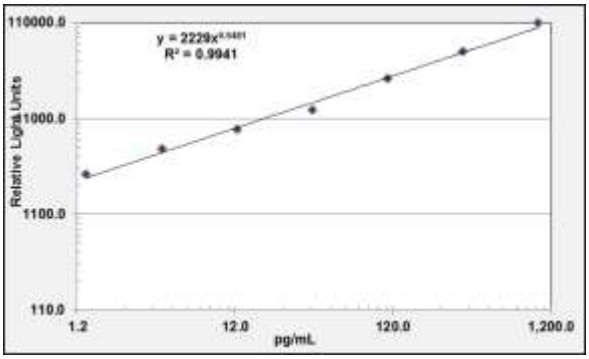
The standard curve is generated by plotting the mean replicate **RLU** of each standard serial dilution point vs. the respective standard concentration. The **AREG** concentration contained in the samples can be interpolated by using linear regression of each mean sample **RLU** against the standard curve. This is best achieved using curve fitting software.

Note: If the samples measured were diluted, multiply the derived mean sample concentration by the dilution factor for a final sample concentration.

12. Typical Expected Data

12.1 Typical standard curve

This standard curve is for demonstration purposes only. An assay specific standard curve should be performed with each assay.



pg/mL	Relative Light Units		Mean RLU	Blank Subtracted
	Rep 1	Rep 2		
1000	109782	109836	109809	109229
333	54287	54665	54476	53896
111	28432	28359	28396	27816
37.04	13498	13372	13435	12855
12.35	8356	8454	8405	7825
4.12	5247	5136	5192	4612
1.37	2842	2859	2851	2271
Blank	574	586	580	NA

12.2 Reproducibility

Intra-assay Precision: 3 samples with known low, middle and high levels AREG were tested with 20 replicates on one plate, respectively. Inter-assay Precision: 3 samples with known low, middle and high level AREG were tested on 3 different plates, 8 replicates in each plate.

Sample	Intra-Assay			Inter-Assay		
	1	2	3	1	2	3
Sample	1	2	3	1	2	3
n	20	20	20	24	24	24
Mean (pg/ml)	18.42	69.35	221.56	17.33	71.25	218.44
SD	0.995	4.092	13.958	0.884	4.346	13.980
CV (%)	5.4	5.9	6.3	5.1	6.1	6.4

12.3 Linearity

Kit linearity evaluated by replicate testing (n=4) serially diluted serum spiked with known concentration of AREG. Results are expressed as the percentage of the expected concentration measurement.

Sample	1:2	1:4	1:8	1:16
Serum (n=5)	83-96%	82-98%	80-103%	84-101%
EDTA Plasma (n=5)	78-95%	85-102%	93-107%	79-93%
Heparin Plasma (n=5)	91-104%	81-91%	86-99%	80-95%

12.4 Recovery

The following matrices were spiked with known concentration of AREG. Recovery is expressed as the percentage of the expected concentration measurement.

Sample Type	Mean Recovery (%)	Range (%)
Serum (n=5)	80 – 93	85
EDTA Plasma (n=5)	85 – 103	96
Heparin Plasma (n=5)	81 – 94	89

13. Technical Resources

Technical Support:

For optimal service please be prepared to supply the lot number of the kit used.

USA

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