# HUMAN ANTERIOR GRADIENT 2 (AGR2) ELISA KIT

**FOR THE QUANTITATIVE DETERMINATION OF HUMAN ANTRIOR GRADIENT 2 (AGR2) CONCENTRATIONS IN SERUM AND PLASMA.**

**PURCHASE INFORMATION:**

| | |
|---|---|
| **ELISA NAME** | **HUMAN ANTERIOR GRADIENT 2 (AGR2) ELISA** |
| **Catalog No.** | **SK00529-01** |
| **Lot No.** | |
| **Formulation** | **96 T** |
| **Standard Range** | **15.6-2000 pg/ml** |
| **Sensitivity** | **3 pg/mL** |
| **Sample Volume** | **100 µl per well** |
| **Sample Type** | **Serum, plasma** |
| **Specificity** | **Human AGR2** |
| **Sample Dilution** | **Optimal dilutions should be determined by each laboratory for each application** |
| **Intra-assay Precision** | **4-6%** |
| **Inter-assay Precision** | **8-12%** |
| **Storage** | **2 °C-8 °C** |

**FOR RESEARCH USE ONLY.NOT FOR USE IN DIAGNOSTIC PROCEDURES.**

**ORDER CONTACT:**
**AVISCERA BIOSCIENCE**
**2348 WALSH AVE., SUITE C**
**SANTA CLARA, CA 95051**
**USA**
**TEL: (408) 982 0300**
**Email: Info@AvisceraBioscience.com**
**Website: www.AvisceraBioscience.com**
**www.AvisceraBioscience.net**

## INTRODUCTION

This Human Anterior Gradient 2 (AGR2) ELISA Kit contains the necessary components required for the quantitative measurement of recombinant and natural human AGR2 from serum and plasma in a sandwich ELISA format.

This immunoassay contains recombinant human AGR2 and antibodies raised against this protein. Results from this immunoassay have shown to accurately quantify natural human AGR2 samples.

## PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for human AGR2 has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any AGR2 present is bound by the immobilized antibody. After washing away any unbound substances, a rabbit antibody specific for human AGR2 is added to wells. Following a wash to remove any unbound detection antibody, an anti rabbit IgG HRP conjugate is added to the wells. Following a wash to remove any unbound enzyme reagent, a substrate solution is added to the wells and color develops in proportion to the amount of AGR2 bound in the initial step. The color development is stopped and the intensity of the color is measured.

## LIMITATIONS OF THE PROCEDURE

_ FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
_ The kit should not be used beyond the expiration date on the kit label.
_ Do not mix or substitute reagents with those from other lots or sources.
_ It is important that the Dilution Buffer selected for the standard curve be consistent with the samples being assayed.
_ If samples generate values higher than the highest standard, dilute the samples with the appropriate Dilution Buffer and repeat the assay.
_ Any variation in standard diluent, operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.
_ This assay is designed to eliminate interference by soluble receptors, binding proteins, and other factors present in biological samples. Until all factors have been tested in the Immunoassay, the possibility of interference cannot be excluded.

## MATERIALS PROVIDED

| DESCRIPTION | CODE | QUANTITY |
|---|---|---|
| **AGR2 Microplate** - 96 well polystyrene microplate (12 strips of 8 wells) coated with monoclonal antibody against human **AGR2**. | 529-01-01 | 1 plate |
| **AGR2 Standard** – 2 ng/vial of recombinant human **AGR2** in a buffered protein base with preservatives; lyophilized. | 529-01-02 | 1 vial |
| **Detection Antibody**– 105 µL / vial, 100-fold concentrated of rabbit antibody against human **AGR2** with preservatives; | 529-01-03 | 1 vial |
| **Positive Control**- one of recombinant human **AGR2**, lyophilized | 529-01-04 | 1 vial |
| **Anti Rabbit IgG HRP Conjugate**- 120 µl /vial, 100-fold of Anti Rabbit IgG HRP conjugate | ARIGHRP | 1 vial |
| **Dilution Buffer**- 60 mL/vial of buffered protein based solution with preservatives | DB06 | 1 vial |
| **HRP Diluent Solution**- 12 mL/vial of buffered protein based solution with preservatives | DB08 | 1 vial |
| **Wash Buffer** -50 ml/vial, 10-fold concentrated buffered surfactant, with preservative. | WB01 | 1 vial |
| **Substrate Solution**-11 ml / vial of TMB substrate solution | TMB01 | 1 vial |
| **Stop Solution** -11 ml /vial of 0.5M HCl | S-STOP | 1 vial |
| **Plate Sealer.** | EAPS | 1 |
| **This kit contains sufficient materials to run 35 samples duplicated provided that assay is run according to protocol.** | | |

## STORAGE

**Unopened Kit:** Store at 2 - 8° C. Do not use past kit expiration date.
**Opened / Reconstituted Reagents: Reconstituted Detection Antibody and Standard Stock** may be stored for up to 1 month at -70°C. Streptavidin HRP conjugate 100 fold concentrated should be stored at 2 - 8° C .
**Microplate Wells:** Return unused wells to the plastic zip bag containing the desiccant pack, reseal along entire edge of zip-seal. May be stored for up to 1 month at 2 - 8° C.

## ADDITIONAL MATERIALS REQUIRED

- Microplate reader capable of absorbance measurement at 450 nm.
- Microplate shaker (250 – 300 rpm).
- Microplate washer or manifold dispenser.
- 100 mL and 500 mL graduated cylinders.
- Multi-channel Pipette, Pipettes and pipette tips.
- Deionized or distilled water.

## PRECAUTION

This kit should be handled by those persons who have been trained in and can follow the principles of good laboratory practice. Wear protective clothing such as laboratory overalls, safety glasses and gloves. Care should be taken while handling solutions in this kit to avoid contact with skin or eyes, especially with the stop solution because it contains diluted hydrochloric acid. Wash immediately with water in case of contact on skin or eyes.

## SAMPLE COLLECTION AND STORAGE

**Serum** – Use a serum separator tube (SST). Allow blood to clot for 30 minutes. Centrifuge at 1000 x g for 15 minutes and collect serum. Assay samples immediately or aliquot and store at ≤ -20° C. Avoid repeated freeze-thaw cycles.
**Plasma** – Collect plasma using EDTA, heparin, or citrate as an anticoagulant. Centrifuge at 1000 x g for 15 minutes and collect plasma. Assay samples immediately or aliquot and store at ≤ -20° C. Avoid repeated freeze-thaw cycles.
**Optional: Use Aprotinin (enzyme inhibitor) (Catalog code: 00070-01-25) for ALL sample collection to prevent sample degradation. 0.5 TIU per mL of sample solution.**

## SAMPLE PREPARATION

Healthy subject serum and plasma samples may not require any dilution.
If the levels of AGR2 in research samples is over 1~ 2 ng/ml, **optimal dilutions should be determined by each laboratory for each application.**

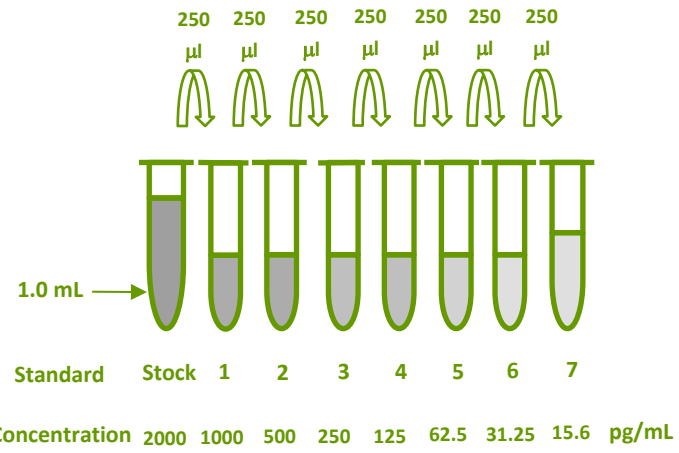
## REAGENT PREPARATION

**Bring all reagents to room temperature before use.**

**Wash Buffer** – If crystals have formed in the concentrate, warm bottle in a water bath until the crystals have completely dissolved. Dilute 50 mL of Wash Buffer Concentrate into 450 mL distilled or deionized water to make 500 mL of 1x Wash Buffer.

**AGR2 Standard** – Reconstitute the human AGR2 standard with 1.0 mL of Dilution Buffer. The concentration of the reconstituted stock solution is 2000 pg/mL. Allow the stock standard to sit for at least 15 minutes with gentle agitation until completely dissolved prior to making standard dilutions (see below). Mix each tube thoroughly before the next transfer. The **2000 pg/mL** standard serves as the high standard. The Dilution Buffer serves as the zero standard (0 pg/mL).

| STANDARD | STANDARD | DILUTION BUFFER | CONCENTRATION |
|---|---|---|---|
| Stock | Powder | 1000 µl | 2000 pg/ml |
| # 1 | 250 µl of stock | 250 µl | 1000 pg/ml |
| # 2 | 250 µl of 1 | 250 µl | 500 pg/ml |
| # 3 | 250 µl of 2 | 250 µl | 250 pg/ml |
| # 4 | 250 µl of 3 | 250 µl | 125 pg/ml |
| # 5 | 250 µl of 4 | 250 µl | 62.5 pg/ml |
| # 6 | 250 µl of 5 | 250 µl | 31.25 pg/ml |
| # 7 | 250 µl of 6 | 250 µl | 15.6 pg/ml |



**Positive Control** - Reconstitute the Positive Control with 1 mL Dilution Buffer. **Note:** Positive Control could be used within a few days if stored at -20° C or -70° C.

**Detection Antibody -** Reconstitute the Detection Antibody with 105 µl Dilution Buffer to prepare 100-fold concentrated stock solution. Pipette 10.395 mL of **Diluent Buffer (DB06)** into a 15 mL centrifuge tube and transfer 105 µL of 100-fold concentrated stock solution to prepare working solution.

## ELISA PROTOCOL

**Bring all reagents and samples to room temperature before the start of the assay. Blank, standard dilutions, positive control and samples should be assayed in duplicate. ELISA Protocol may need further optimization.**

1. Prepare all reagents, standard dilutions, positive control and samples as directed previously.
2. Remove unneeded microplate strips from the plate frame and return them to the plastic pouch with the desiccant pack.
3. Add 100 µL per well of **Dilution Buffer** to Blank wells (A2, A3).
4. Add 100 µL per well of **Standard Dilutions** in reverse order of serial dilution from #7-S (B2, B3 to G2, G3, from G4, G5 to F4, F5), **sample,** or **positive control** (C4, C5). Cover with plate sealer and incubate for 2 hours on microplate shaker at room temperature.
5. Aspirate and wash each well with 300 µL of **1x Wash Buffer** four times. After the last wash, aspirate any remaining 1x Wash Buffer, invert the plate and blot against clean paper towel(s).
6. Add 100 µL per well of **Detection Antibody working solution**. Cover with plate sealer and incubate for 2 hour on microplate shaker at room temperature.
7. Repeat the aspiration and wash as in step 5.
8. Add 100 µL per well of **Anti Rabbit IgG HRP conjuagte**. Cover with plate sealer and incubate for 1 hour on microplate shaker at room temperature. **Protect from light.**
9. Repeat the aspiration and wash as in step 5.
10. Add 100 µL per well of **Substrate Solution**. Incubate for 15-18 minutes on microplate shaker at room temperature. **Protect from light.**
11. Add 100 µL per well of **Stop Solution**. The color in the wells should change from blue to yellow. If the color change does not appear uniform, gently tap the plate to ensure thorough mixing.
12. Read plate using a microplate reader set to 450 nm within 15 minutes.

## CALCULATION OF RESULTS

Create a standard curve by plotting the log of the known concentrations of the standard dilutions (x-axis) versus the log of its corresponding O.D. (y-axis) and draw the best fit line through the points. It is recommended to use computer software capable of generating a log-log curve fit to more accurately quantify the standard dilutions.

If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

## CALIBRATION

This immunoassay is calibrated against a highly purified recombinant human AGR2.

## SENSITIVITY

Twenty-five assays were evaluated and the minimum detectable dose (MDD) of AGR2 was 3 pg/mL.

## TYPICAL DATA

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

| STANDARD (PG/ML) | CORRECTED (450NM) |
|---|---|
| Blank | 0 (0.058) |
| 15.6 | 0.036 |
| 31.25 | 0.073 |
| 62.5 | 0.147 |
| 125 | 0.314 |
| 250 | 0.558 |
| 500 | 1.003 |
| 1000 | 1.800 |
| 2000 | 2.624 |

## SPECIFICITY

| PROTEINS | CROSS-REACTIVITY(%) |
|---|---|
| **Human AGR2** | 100 |
| **Human AGR1** | 0 |
| **Human AGR3** | 0 |
| **Human HE4** | 0 |
| **Human sHer2** | 0 |

## SUMMARY OF ASSAY PROCEDURE

| **PREPARE REAGENTS, SAMPLES AND STANDARD DILUTIONS** |
|---|
| ↓ |
| Add 100 µL of standard dilutions, samples and positive control. Cover with plate sealer and incubate 2 hours on microplate shaker at RT. |
| ↓ |
| Aspirate and wash 4 times. |
| ↓ |
| Add 100 µL per well of Detection Antibody working solution. Cover with plate sealer and incubate 2 hours on microplate shaker at RT. |
| ↓ |
| Aspirate and wash 4 times. |
| ↓ |
| Add 100 µL per well of Anti Rabbit IgG HRP conjugate working solution. Cover with plate sealer and incubate 1 hour on microplate shaker at RT. **Protect from light.** |
| ↓ |
| Aspirate and wash 4 times. |
| ↓ |
| Add 100 µL per well of Substrate Solution. Incubate 15-18 min on microplate shaker at RT. **Protect from light.** |
| ↓ |
| Add 100 µL per well of Stop Solution. Read at 450 nm within 15 minutes. |