HUMAN ANTERIOR GRADIENT PROTEIN 2 (AGR2) ELISA KIT

FOR THE QUANTITATIVE DETERMINATION OF HUMAN AGR2 CONCENTRATIONS IN SERUM AND PLASMA

ALWAYS REFER TO LOT SPECIFIC PROTCOL PROVIDED WITH EACH KIT FOR INSTRUCTIONS. PROTOCOL MUST BE READ BEFORE USING THIS PRODUCT.

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

PRODUCT INFORMATION:

THIS KIT IS FOR ONE TIME USE ONLY.

ELISA NAME	AGR2 (HUMAN) ELISA KIT
Catalog No.	SK00529-02
Lot No.	
Formulation	96 T
Standard range	39-2500 pg/mL
Sensitivity	20 pg/mL
Sample Volume	100 µL
Sample Type	Serum, Plasma
Pretreatment	*require*
Specificity	Human AGR2
Calibration	Human AGR2 recombinant
Intra-assay Precision	4 - 6%
Inter-assay Precision	8 - 10%
Storage	2 - 8° C for 1 month, see page 2-3 for more information
This kit contains sufficient materials to run approximately 35 samples duplicated provided that assay is run according to protocol.	

ORDER CONTACT:
AVISCERA BIOSCIENCE, INC.
2348 Walsh Ave., Suite C
Santa Clara, CA 95051
USA
Tel: (408) 982 0300
Fax: (408) 982 0301
Email: Sales@AvisceraBioscience.com
Info@AvisceraBioscience.com
www.AvisceraBioscience.net
www.AvisceraBioscience.com

DESCRIPTION

This Human Anterior Gradient Protein 2 (AGR2) ELISA Kit contains the necessary components required for the quantitative measurement of recombinant and/or natural AGR2 from serum samples 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 recombinant and natural human AGR2 samples.

ASSAY OVERVIEW

This assay employs the quantitative sandwich enzyme immunoassay technique. The plate is pre-coated with a polyclonal antibody specific for human AGR2. The capture antibody can bind to the human AGR2 in the standard and samples. After washing the plate of any unbound substances, a biotinylated monoclonal antibody against human AGR2 is added to the wells. After another washing of the plate, Streptavidin-HRP Conjugate is added. After the last wash to remove any unbound enzyme, a substrate solution is added to the wells and color develops in direct proportion to the amount of human AGR2 bound in the standard solutions or samples. A standard curve can be established and sample values can be read off the standard curve.

PROCEDURAL LIMITATIONS

_FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
_This ELISA kit should not be used beyond the expiration date on the kit label.
_Do not mix 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.
_Any modifications in buffers, pipetting technique, washing technique, incubation time or temperature, as well as kit age can cause a change in signal.
_Not all interfering factors have been tested in the immunoassay, therefore the possibility of interference cannot be excluded.

COMPONENTS PROVIDED

DESCRIPTION	CODE	QUANTITY
AGR2 Microplate - 96 well polystyrene microplate (12 strips of 8 wells) coated with an antibody against human AGR2.	**529-02-01**	**1 plate**
Human AGR2 Standard – refer to lot of recombinant human AGR2 in a buffered protein base with preservative; lyophilized.	**529-02-02**	**1 vial**
Detection Antibody Concentrate – refer to lot vial of biotinylated monoclonal antibody against human AGR2 with preservative	**529-02-03**	**1 vial**
Positive Control –one vial, of human AGR2 recombinant with preservative	**529-02-04**	**1 vial**
Streptavidin-HRP Conjugate - 120 µl/vial, 100-fold concentrated solution of Streptavidin conjugate to HRP.	**SAHRP**	**1 vial**
Dilution Buffer – 40 mL of buffered protein based solution with preservative.	**DB06**	**1 bottle**
HRP Diluent Solution – 12 mL of buffered protein based solution with preservative.	**DB08A**	**1 bottle**
Wash Buffer - 50 mL of 10-fold concentrated buffered surfactant, with preservative.	**WB01**	**1 bottle**
TMB Substrate Solution - 11 mL of TMB substrate solution.	**TMB01**	**1 bottle**
Stop Solution - 11 mL of 0.5M HCl solution.	**S-STOP**	**1 bottle**
Plate Sealer	**EAPS**	**1 piece**
Plastic Pouch	**P01**	**1 piece**

STORAGE

Unopened Kit: Store at 2 – 8° C for up to 1 month. For longer storage up to 10 months, unopened Standard, Dilution Buffer and HRP Diluent Solution should be stored at -20° C. Streptavidin-HRP Conjugate and TMB Substrate Solution should be stored only at 2-8° C. Do not use kit past expiration date.

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.
- 500 mM TCEP
- 0.1% SDS in PBS, pH 7.4

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) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at ≤ -20° C. Avoid repeated freeze-thaw cycles.
Plasma - Collect plasma using EDTA as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20° C. Avoid repeated freeze-thaw cycles.
Optional: Use Aprotinin (enzyme inhibitor) for ALL sample collection to prevent sample degradation. 0.5 TIU per ml of sample solution

SAMPLE PREPARATION

*Due to dimer of AGR2 in circulating samples, serum or EDTA plasma samples NEED to be pretreated before being added to the microplate. Standard and Positive Control **DO NOT NEED** to be treated.*

1. Add 240 µl of 500 mM TCEP to 11.76 mL of Sample Buffer **(STB01)** to prepare the Pretreatment Solution (10mM TCEP, 0.1% SDS in PBS, pH 7.4) (12 mL for 50 samples pretreatment).
2. Add 70 µl of sample to 210 µl of Pretreatment Solution in a polypropylene tube or vial. **Note: This pretreatment dilution (4-fold dilution) may require optimization.**
3. Vortex gently and incubate for 30 minutes at room temperature. Assay immediately and discard any excess pretreated samples.
Optimal dilutions should be determined by each laboratory for each application with a sample pretest.
Use polypropylene test tubes.

REAGENT PREPARATION

Bring all reagents to room temperature before use.
Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Dilute 50 mL of Wash Buffer Concentrate into deionized or distilled water (450 mL) to prepare 500 mL of 1x Wash Buffer.

AGR2 Standard - Reconstitute the AGR2 standard with refer to lot of Dilution Buffer. Pipette 250 µL of Dilution Buffer into the tube #2 to #6. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The **2500 pg/mL** standard serves as the high standard. The Dilution Buffer serves as the zero standard (0 pg/mL).

TUBE	STANDARD	DILUTION BUFFER	CONCENTRATION
stock	powder	Refer to lot	Refer to lot
# 1	Refer to lot	Refer to lot	2500 pg/ml
# 2	250µl of 1	250µl	1250 pg/ml
# 3	250µl of 2	250µl	625 pg/ml
# 4	250µl of 3	250µl	312.5 pg/ml
# 5	250µl of 4	250µl	156 pg/ml
# 6	250µl of 5	250µl	78 pg/ml
# 7	250µl of 6	250µl	39 pg/ml

Positive Control - Reconstitute the Positive Control with refer to lot of Dilution Buffer.

Detection Antibody - Reconstitute the Detection Antibody Concentrate with refer to lot of Dilution Buffer to prepare 10-fold concentrated stock. Pipette 9.45 mL of Dilution Buffer into a 15 ml centrifuge tube and transfer 1.05 mL of 10-fold concentrated stock solution to prepare working solution.

Streptavidin-HRP Conjugate - Pipette 11.88 mL of **HRP Diluent solution (DB08A)** into a 15 mL centrifuge tube and transfer 120 µL of 100-fold concentrated stock solution to prepare working solution (**protect from light**).

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 and working standards as directed in the previous sections.
2. Add 100 µL per well of Dilution Buffer to Blank wells.
3. Add 100 µL of standard dilutions in reverse order of serial dilution, samples, or positive control per well. Cover with plate sealer. Incubate for 2 hours on microplate shaker at room temperature.
4. Aspirate each well and wash, repeating the process three times for a total of four washes. Wash by filling each well with 1x Wash Buffer (300 µL) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
5. Add 100 µL of Detection Antibody working solution to each well. Cover with plate sealer. Incubate for 2 hours on microplate shaker at room temperature.
6. Repeat the aspiration/wash as in step 4.
7. Add 100 µL of Streptavidin-HRP Conjugate working solution to each well. Incubate for 60 minutes on microplate shaker at room temperature. **Protect from light.**
8. Repeat the aspiration/wash as in step 4.
9. Add 100 µL of Substrate Solution to each well. Incubate for refer to lot on microplate shaker at room temperature. **Protect from light.**
10. Add 100 µL of Stop Solution to each well. The color in the wells should change from blue to yellow. If the color in the wells is green, or if the color change does not appear uniform, gently tap the plate to ensure thorough mixing.
11. Determine the optical density of each well using a microplate reader set to 450 nm.

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.

SPECIFICITY

PROTEINS	CROSS-REACTIVITY (%)
Human AGR2	100
Human AGR3	0

TYPICAL STANDARD CURVE

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

STANDARD (PG/ML)	AVERAGE OD450 (CORRECTED)
Blank	0 (refer to lot)
39	0.072
78	0.120
156	0.224
312.5	0.428
625	0.760
1250	1.882
2500	2.580

SUMMARY OF ASSAY PROCEDURE

PREPARE REAGENTS, SAMPLES AND STANDARDS
⬇
Add 100 µl of standard dilutions, samples, or positive control to the well. Incubate 2 hours on the plate shaker at RT.
⬇
Aspirate and wash 4 times.
⬇
Add 100 µl Detection Antibody working solution to each well. Incubate 2 hours on the plate shaker at RT.
⬇
Aspirate and wash 4 times.
⬇
Add 100 µl Streptavidin-HRP conjugate working solution to each well. Incubate 60 min on the plate shaker at RT. **Protect from light**.
⬇
Aspirate and wash 4 times.
⬇
Add 100 µl Substrate solution to each well. Incubate refer to lot on the plate shaker at RT. **Protect from light**.
⬇
Add 100 µl Stop Solution to each well. Read at 450 nm.