Zinc-α-2-glycoprotein (human) Matched Pair Detection Set

*[*ZAG (human) *Matched Pair Detection Set]*

Cat. N° AG-46B-0008-KI01 *For Research Use Only* *11-May-2015*

1. Intended Use

The Zinc-α-2-glycoprotein (human) Matched Pair Detection Set contains key reagents required to develop a sandwich ELISA to measure human Zinc-α-2-glycoprotein. The assay procedure has been optimized for cell culture supernatant, serum and plasma.

2. Materials Provided

• AG-46B-0008/STD	Standard	1 vial	(1 µg) (*lyophilized*)	(STD)
• AG-46B-0008/COAT	Coating Antibody	1 vial	(120 µl)	(COAT)
• AG-46B-0008/DET	Detection Antibody	1 vial	(60 µl)	(DET)
• AG-46B-0008/STREP	Streptavidin-HRP	1 vial	(10 µg) (*lyophilized*)	(STREP)

3. Materials Required

- PBS: 137mM NaCl, 2.7mM KCl, 8.1mM Na_2HPO_4, 1.5mM KH_2PO_4, pH 7.2, 0.2µM filtered
- Wash Buffer: 0.1% Tween® 20 in PBS
- Blocking Buffer: 2% BSA (ELISA grade) in PBS, 0.2µM filtered
- ELISA Buffer: 0.2% BSA (ELISA grade) and 0.05% Tween® 20 in PBS
- TMB: Tetramethylbenzidine
- Plates: Nunc-Immuno MaxiSorp, N°467466
- Stop Solution: 2M sulphuric acid (H_2SO_4)
- Distilled or deionized water

4. Product Specifications

- Number of Assays: Contains sufficient materials to run ELISAs on 5 x 96-well plates.
- Range: 0.0156 ng/ml to 1 ng/ml
- Specificity: Recognizes human Zinc-α-2-glycoprotein.
- Stability: Stable at least 1 year after receipt when stored at -20°C.

5. General ELISA Protocol

a) Reagent Preparation

- Dilute the desired amount of Coating Antibody (COAT) (1mg/ml) (mouse monoclonal antibody) to 2µg/ml in PBS without carrier protein and use it fresh.

- Dilute the desired amount of Detection Antibody (DET) (0.1mg/ml) (biotinylated mouse monoclonal antibody) to 0.1µg/ml in ELISA Buffer and use it fresh.

- Reconstitute the Streptavidin-HRP (STREP) with 100µl ELISA Buffer. After reconstitution, prepare aliquots and store the reconstituted STREP at -20°C. Avoid freeze/thaw cycles. Dilute the reconstituted Streptavidin-HRP to the working concentration by adding 5µl in 10ml of ELISA Buffer (1:2000).

- Reconstitute the Standard Protein (STD) (recombinant human Zinc-α-2-glycoprotein) with 100µl ELISA Buffer to obtain a concentration of 10µg/ml. After reconstitution, prepare aliquots and store the reconstituted standard at -20°C. Avoid freeze/thaw cycles. A standard curve using 2-fold serial dilutions in ELISA Buffer is recommended. Suggested standard points are 1ng/ml, 0.5ng/ml, 0.25ng/ml, 0.125ng/ml, 0.062ng/ml, 0.031ng/ml, 0.0156ng/ml and 0ng/ml.

- **Cell supernatants, serum and plasma** have to be diluted in ELISA Buffer 1X. *As a starting point, 1/200'000 to 1/400'000 dilutions of serum of plasma are recommended! If sample values fall outside the detection range of the assay, a lower or higher dilution may be required!*

Note that diluted reagents described in this section are not stable and cannot be stored.

b) Plate Preparation

1. Coat the wells by adding 100µl/well of diluted Coating Antibody (2µg/ml) (see section Reagent Preparation) to a 96-well ELISA microplate (Nunc MaxiSorp™ flat-bottom 96 well plate is suggested). Cover the plate with plastic film and leave 2h at 25°C.

2. Aspirate the coated wells and add 300µl of Wash Buffer using a multichannel pipette or autowasher. Repeat the process for a total of five washes. Complete removal of liquid at each step is essential for good performance. After the last wash, remove any remaining Wash Buffer by inverting the plate and blotting it against clean absorbent paper.

3. Block plates by adding 200µl of Blocking Buffer for 2h at 25°C.

4. Aspirate the coated wells and add 300µl of Wash Buffer using a multichannel pipette or autowasher. Repeat the process for a total of five washes. Complete removal of liquid at each step is essential for good performance. After the last wash, remove any remaining Wash Buffer by inverting the plate and blotting it against clean absorbent paper.

c) Assay Procedure

1. Add a total of 100µl/well of cell culture supernatant, plasma or serum (diluted in ELISA Buffer) or standard protein (see section Reagent Preparation) to the plate.
2. Cover the plate with plastic film and incubate for 2h at room temperature.
3. Repeat the aspiration/wash as in step 4 of Plate preparation.
4. Add 100µl/well of the diluted Detection Antibody (0.1µg/ml) (see section Reagent Preparation).
5. Cover the plate with plastic film and incubate for one hour at room temperature.
6. Repeat the aspiration/wash as in step 4 of Plate preparation.
7. Add 100µl to each well of the diluted Streptavidin-HRP (see section Reagent Preparation).
8. Cover the plate with plastic film and incubate for 30min at room temperature.
9. Repeat the aspiration/wash as in step 4 of Plate preparation.
10. Substrate development is conducted by addition of 100µl to each well of ready-to-use TMB for 10-20min at room temperature.
11. Stop the reaction by adding 50µl of Stop Solution (2M H_2SO_4). Tap the plate gently to ensure thorough mixing.
12. Measure the OD at 450nm in an ELISA reader.
13. Measure absorbance at 550nm and subtract these values from those obtained at 450nm to correct for optical imperfections in the microplate. If absorbance at 550nm is not possible, measure the absorbance at 450nm only.
    Note: When the 550nm measurement is omitted, absorbance values will be higher.

6. Technical Hints

- Once reagents have been added to the plate, DO NOT let the plate dry at any time during the assay.
- It is recommended that all standards and samples be assayed in duplicate.
- Avoid microbial contamination of reagents and equipment. Automated plate washers can become contaminated thereby causing assay variability. Buffers containing a large quantity of proteins should be made under sterile conditions and stored at 2-8°C or be prepared fresh daily.
- Vigorous plate washing is essential.
- Avoid exposing reagents to excessive heat or light during storage and incubation.
- Wear gloves while performing the assay to avoid contact with samples and reagents. Please follow proper disposal procedure.
- The Stop Solution (STOP) consists of sulfuric acid. Although diluted, the Stop Solution should be handled with gloves, eye protection and protective clothing.

7. Calculation of Results

- Average the duplicate readings for each standard, control and sample and subtract the average blank value.

- Generate the standard curve by plotting the average absorbance obtained for each standard concentration on the vertical (Y) axis vs. the corresponding human Zinc-α-2-glycoprotein concentration (µg/ml) on the horizontal axis (see 8. Expected Standard Curve). Calculate results using graph paper or curve-fitting statistical software. The amount of human Zinc-α-2-glycoprotein in each sample is determined by interpolating for the absorbance value (Y axis) using the standard curve.

- If the test samples were diluted, multiply the interpolated values by the dilution factor to calculate µg/ml of human Zinc-α-2-glycoprotein in the samples.

8. Expected Standard Curve

The following curve is obtained using the different concentrations of standard as described in this protocol:

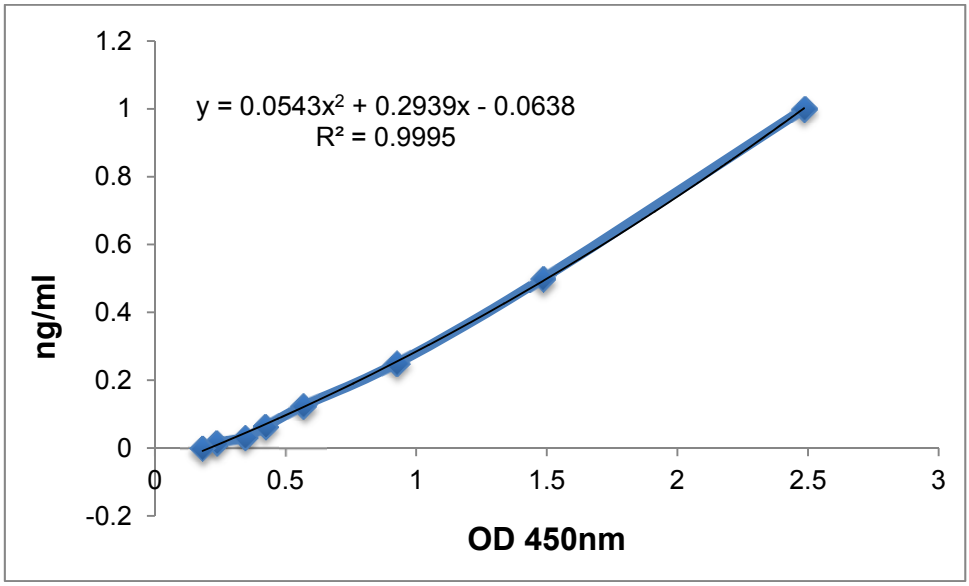

Figure: Standard Curve

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