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User's Manual

Human Zinc-Alpha-2-Glycoprotein ELISA Kit

REF **DEIA-WZ131**

Σ **96T**

RUO

This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

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PRODUCT INFORMATION

Intended Use

The Human Zinc-Alpha-2-Glycoprotein ELISA is a sandwich enzyme immunoassay for the quantitative measurement.

of human zinc-alpha-2-glycoprotein in serum and plasma

General Description

Zinc-alpha-2-glycoprotein (ZAG, ZA2G, Azgp1, ZNGP1, Lipid-Mobilizing Factor, LMF) is a soluble 41kDa glycoprotein belonging to the immunoglobulin protein family and consisting of a single polypeptide chain. Human ZAG shares 59% sequence identity with the murine homolog. ZAG is closely related to antigens of the class1 major histocompatibility complex (MHC I) and shares 30-40% sequence identity with the heavy chain of MHC I. Most MHC-I members heterodimerize with beta-2-microglobuline (b2m) and bind peptides derived from intracellular proteins to present them to cytotoxic T cells. In contrast, ZAG is a soluble protein rather than being anchored to plasma membranes that acts independently on b2m and binds the hydrophobic ligand, which may relate to its function in lipid metabolism. ZAG is widespread in body fluids and is also found in various human tissues such as adipose tissue, prostate, breast, skin, salivary gland, trachea, bronchus, lung, gastrointestinal tract, pancreas, liver and kidney. ZAG acts as a lipid mobilizing factor to induce lipolysis in adipocytes and plays an important role in lipid utilization and loss of adipose tissue, especially during cachexia, which occurs in patient suffering from cancer, AIDS and other chronic illnesses. The role of ZAG in cancer cachexia is also connected with its ability to directly influence expression of uncoupling proteins (UCPs), which are implicated in the regulation of energy balance. In human adipocytes, ZAG expression is regulated particularly through TNF alpha and the PPAR gamma nuclear receptor. ZAG expression is also upregulated by glucocorticoids and attenuated by eicosatetraenoic acid (EPA) and beta-3-adrenoreceptor antagonists. ZAG is overexpressed in certain human malignant tumors such as prostate, breast, lung or bladder cancer and can relate to tumor differentiation. Additionally, ZAG plays a role in obesity, diabetic kidney disorders, frontotemporal dementia and regulation of melanin production by melanocytes. ZAG is proposed to have a therapeutic use in obesity and cachexia. It can be used as a marker for clinical analysis of diabetic nephropathy and as a marker for certain tumors.

Principles of Testing

In the Human Zinc-Alpha-2-Glycoprotein ELISA Kit, standards, quality controls and samples are incubated in microplate wells pre-coated with polyclonal anti-human ZA2G antibody. After 60 minutes incubation and washing, polyclonal anti-human ZA2G antibody, conjugated with horseradish peroxidase (HRP) is added to the wells and incubated for 60 minutes with captured ZA2G. Following another washing step, the remaining HRP conjugate is allowed to react with the substrate solution (TMB). The reaction is stopped by addition of acidic solution and absorbance of the resulting yellow product is measured. The absorbance is proportional to the concentration of ZA2G. A standard curve is constructed by plotting absorbance values against concentrations of standards, and concentrations of unknown samples are determined using this standard curve.

Reagents And Materials Provided

1. Antibody Coated Microtiter Strips, ready to use, 96 wells
2. Conjugate Solution, ready to use, 13 ml
3. Master Standard, lyophilized, 1 vial
4. Quality Control HIGH, lyophilized, 1 vial
5. Quality Control LOW, lyophilized, 1 vial
6. Dilution Buffer Conc. (2x), concentrated, 50 ml
7. Wash Solution Conc. (10x), concentrated, 100 ml
8. Substrate Solution, ready to use, 13 ml
9. Stop Solution, ready to use, 13 ml
10. Product Data Sheet + Certificate of Analysis - 1 pc

Materials Required But Not Supplied

• Deionized (distilled) water

• Test tubes for diluting samples

• Glassware (graduated cylinder and bottle) for Wash Solution (Dilution Buffer)

• Precision pipettes to deliver 5-1000 µl with disposable tips

• Multichannel pipette to deliver 100 µl with disposable tips

• Absorbent material (e.g. paper towels) for blotting the microtitrate plate after washing

• Vortex mixer

• Orbital microplate shaker capable of approximately 300 rpm

• Microplate washer (optional).

• Microplate reader with 450 ± 10 nm filter, preferably with reference wavelength 630 nm (alternatively another one from the interval 550-650 nm)

• Software package facilitating data generation and analysis (optional)

Storage

2-8°C

Assay Procedure

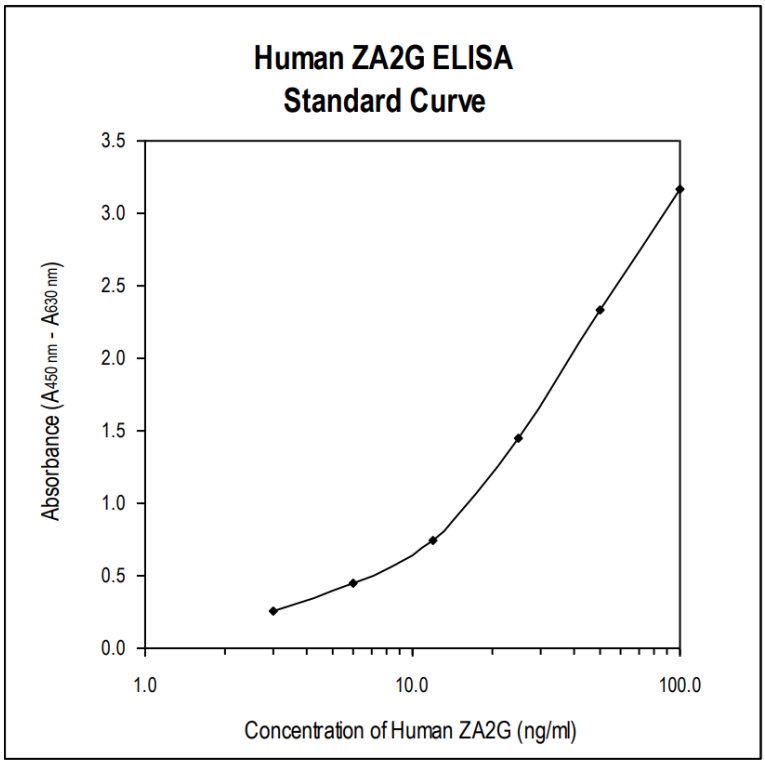
1. Pipet 100 µl of diluted Standards, Quality Controls, Dilution Buffer (=Blank) and samples, preferably in duplicates, into the appropriate wells. See Figure 1 for example of work sheet.
2. Incubate the plate at room temperature (ca. 25°C) for 1 hour, shaking at ca. 300 rpm on an orbital microplate shaker.
3. Wash the wells 3-times with Wash Solution (0.35 ml per well). After final wash, invert and tap the plate strongly against paper towel.
4. Add 100 µl of Conjugate Solution into each well.

5. Incubate the plate at room temperature (ca. 25°C) for 1 hour, shaking at ca. 300 rpm on an orbital microplate shaker.
6. Wash the wells 3-times with Wash Solution (0.35 ml per well). After final wash, invert and tap the plate strongly against paper towel.
7. Add 100 µl of Substrate Solution into each well. Avoid exposing the microtiter plate to direct sunlight. Covering the plate with e.g. aluminium foil is recommended.
8. Incubate the plate for 10 minutes at room temperature. The incubation time may be extended [up to 20 minutes] if the reaction temperature is below than 20°C. Do not shake the plate during the incubation.
9. Stop the colour development by adding 100 µl of Stop Solution.
10. Determine the absorbance of each well using a microplate reader set to 450 nm, preferably with the reference wavelength set to 630 nm (acceptable range: 550 – 650 nm). Subtract readings at 630 nm (550 - 650 nm) from the readings at 450 nm. The absorbance should be read within 5 minutes following step 9.

Note 1: If some samples and standard/s have absorbances above the upper limit of your microplate reader, perform a second reading at 405 nm. A new standard curve, constructed using the values measured at 405 nm, is used to determine ZA2G concentration of off-scale standards and samples. The readings at 405 nm should not replace the readings for samples that were "in range" at 450 nm.

Note 2: Manual washing: Aspirate wells and pipet 0.35 ml Wash Solution into each well. Aspirate wells and repeat twice. After final wash, invert and tap the plate strongly against paper towel. Make certain that Wash Solution has been removed entirely.

Typical Standard Curve



Precision

Intra-assay CV≤ 3.9%

Inter-assay CV≤ 6.6%

Sensitivity

0.673 ng/ml.

Specificity

The antibodies used in this ELISA are specific for human ZA2G with no detectable crossreactivities to human leptin, resisitin, ANGPTL4, visfatin at 50 ng/ml and human HLA-G at 250 U/ml.

Recovery

96.2%