

#### RayBiotech, Inc.

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# Certificate of Analysis and Data Sheet

# Recombinant Human Zinc-alpha-2 Glycoprotein (ZAG) / AZGP1

**Catalog No.** 228-11659

Source

293 Cell Line (Human Embryonic Kidney)

## **Synonyms**

Zn-alpha-2-glycoprotein, Zn-alpha-2-GP, AZGP1, ZAG, Zinc-alpha-2-glycoprotein, ZNGP1, ZA2G. Zinc-alpha-2-glycoprotein (ZAG) is found in body fluids such as serum, sweat, and seminal and breast cyst fluids. It is identical in amino acid sequence to tumor-derived lipid mobilizing factor (LMF), a protein associated with the dramatic loss of adipose body stores in cancer cachexia, and has been shown to stimulate lipolysis by adipocytes in vivo and in vitro. A role for ZAG has been proposed in the regulation of body weight, and age-dependent changes in genetically influenced obesity, and also it regulates melanin production by normal and malignant melanocytes. It has also recently been classified as a novel adipokine in that it is produced by both white and brown fat adipocytes and may act in a local autocrine fashion in the reduction of adiposity in cachexia. Controlling ZAG/LMF's activity could be life-saving in the management of certain cancers and other cachexia inducing conditions, and its possible normal role in body fat store homeostasis is deserving of understanding in its own right. ZAG exhibits a class I major histocompatibility complex (MHC) fold but is a soluble protein rather than being anchored to plasma membranes and does not associate with alpha-2-microglobulin in humans. Like antigen-presenting MHC class I proteins, ZAG has an open apical groove, and X-ray crystallography of human derived ZAG revealed an unidentifiable electron density in a similar position to that occupied by antigenic peptides in classical MHC proteins and glycolipids in isoforms of CD1. This presumptive ligand is not a peptide, and the groove is too small to hold a glycolipid such as is presented by CD1 isoforms. By analogy with all other MHC class I-related proteins that have an open apical groove (some do not), occupancy by a ligand is probably crucial to ZAG's biological function. Despite all of the structural and biochemical evidence that ZAG binds a ligand, none has so far been found by extraction from protein isolated from biological fluids. This difficulty could be because the ligand is labile, heterogeneous, or readily lost during purification procedures. Knowing more about how ZAG interacts with the compounds it has been found to bind, both natural and artificial, will inform searches for the elusive ligand(s) and its/their role in ZAG's signaling function.

# Description

ZA2G Human Recombinant produced in HEK cells is a single, glycosylated polypeptide chain containing a total of 290 amino acids encoding (13-290). ZA2G Human Recombinant is identical to Swiss-Prot-P25311 (AA 18-295, mature Zinc-Alpha-2-Glycoprotein). Twelve extra amino acids were fused with the N-terminus (bold).



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ASWSHPQFEK GSQENQDGRY SLTYIYTGLS KHVEDVPAFQ ALGSLNDLQF FRYNSKDRKS QPMGLWRQVE GMEDWKQDSQ LQKAREDIFM ETLKDIVEYY NDSNGSHVLQ GRFGCEIENN RSSGAFWKYY YDGKDYIEFN KEIPAWVPFD PAAQITKQKW EAEPVYVQRA KAYLEEECPA TLRKYLKYSK NILDRQDPPS VVVTSHQAPG EKKKLKCLAY DFYPGKIDVH WTRAGEVQEP ELRGDVLHNG NGTYOSWVVV AVPPODTAPY SCHVOHSSLA OPLVVPWEAS.

## Physical Appearance

Filtered White lyophilized (freeze-dried) powder.

#### **Formulation**

Filtered (0.4 µm) and lyophilized in 0.5 mg/ml in 0.1M Tris-HCl pH 8.0 and 150mM NaCl.

#### Solubility

Add deionized water to a working concentration approximately 0.5 mg/ml and let the lyophilized pellet dissolve completely.

## Stability

Lyophilized ZA2G although stable at room temperature for 3 weeks, should be stored desiccated below -18°C. Upon reconstitution ZA2G should be stored at 4°C between 2-7 days and for future use below -18°C. For long term storage it is recommended to add a carrier protein (0.1% HSA or BSA). Please avoid freeze-thaw cycles.

# Purity

Greater than 90.0% as determined by

- (a) Analysis by RP-HPLC.
- (b) Analysis by SDS-PAGE.

#### Biological Activity

Differentiated human SGBS adipocytes were incubated for 18 h at two dose levels of rhZA2G - 5 and 20  $\mu g/ml$ . Lipolysis was quantified by measuring glycerol release into the medium using a standard protocol. Isoproterenol (10  $\mu M$ ) and IBMX (100  $\mu M$ ) were used as positive controls. "Con" stands for the negative control. There was a 3-fold increase in glycerol release with both doses. The increase was statistically significant at 5  $\mu g/ml$  dose of rhZA2G (p<0.01) as well as in positive controls.