3050 Spruce Street, St. Louis, MO 63103 USA
Tel: (800) 521-8956 (314) 771-5765 Fax: (800) 325-5052 (314) 771-5757
email: techservice@sial.com sigma-aldrich.com

Product Information

Zinc-binding α-2 glycoprotein (ZAG) EIA Kit
for serum, plasma, culture supernatant, and cell lysates

Catalog Number **RAB0023**
Storage Temperature –20 °C

TECHNICAL BULLETIN

Product Description

The Zinc-binding α-2 glycoprotein (ZAG) Enzyme Immunoassay (EIA) Kit is an *in vitro* quantitative assay for detecting ZAG peptide based on the principle of competitive enzyme immunoassay. The microplate in the kit is pre-coated with anti-rabbit secondary antibody. After a blocking step and incubation of the plate with anti-ZAG antibody, both biotinylated ZAG peptide, and peptide standard or targeted peptide in samples interacts competitively with the ZAG antibody. Uncompeted (bound) biotinylated ZAG peptide then interacts with Streptavidin-horseradish peroxidase (SA-HRP), which catalyzes a color development reaction. The intensity of colorimetric signal is directly proportional to the amount of biotinylated peptide-SA-HRP complex and inversely proportional to the amount of ZAG peptide in the standard or samples. This is due to the competitive binding to ZAG antibody between biotinylated ZAG peptide and peptides in standard or samples. A standard curve of known concentration of ZAG peptide can be established and the concentration of ZAG peptide in the samples can be calculated accordingly.

Components

1. 96-well plate coated with secondary antibody (Item A) - RAB0023A: 96 wells (12 strips × 8 wells) coated with secondary antibody.
2. 20x Wash Buffer (Item B) - RABWASH3: 25 mL
3. EIA Zinc-binding alpha-2 glycoprotein Peptide standard (Item C) - RAB0023C: 2 vials, 10 mL/vial
4. Anti-Zinc-binding α-2 glycoprotein (ZAG) Detection Antibody (Item N) - RAB0023F: 2 vials, 5 mL/vial
5. EIA Assay Diluent A (Item D) - RABDIL9: 30 mL, contains 0.09% sodium azide as preservative. Diluent for standards, and serum or plasma samples.
6. EIA 5x Assay Diluent B (Item E) - RABDIL10: 15 mL of 5x concentrated buffer. Diluent for standards and cell culture media or other sample types.
7. Biotinylated Zinc-binding α-2 glycoprotein (ZAG) Peptide (Item F) - RAB0023G: 2 vials, 20 mL/vial
8. 100x HRP-streptavidin (Item G) - RABHRP3: 200 µL of 100x concentrated HRP-conjugated Streptavidin.
9. Zinc-binding α-2 glycoprotein (ZAG) Positive Control Sample, Lyophilized (Item M) - RAB0023K: 1 vial, 100 mL
10. TMB Substrate solution (Item H) - RABTMB2: 12 mL of 3,3′,5,5′-tetramethylbenzidine (TMB) in buffered solution
11. Stop Solution (Item I) – RABSTOP3: 8 mL of 0.2 M sulfuric acid.

Reagents and Equipment Required but Not Provided.

1. Microplate reader capable of measuring absorbance at 450 nm.
2. Precision pipettes to deliver 2 µL to 1 mL volumes.
3. Adjustable 1-25 mL pipettes for reagent preparation.
4. 100 mL and 1 liter graduated cylinders.
5. Absorbent paper.
6. Distilled or deionized water.
7. Log-log graph paper, or computer and software for ELISA data analysis.
8. Tubes to prepare standard or sample dilutions.
9. Orbital shaker.
10. Aluminum foil.
11. Saran wrap.

Precautions and Disclaimer

For R&D use only. Not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices

Preparation Instructions

If testing plasma or serum samples, use Assay Diluent A to dilute Item F and Item C. If testing cell culture media or other sample types, use Assay Diluent B to dilute Item F and Item C. For sample and positive

control dilutions, refer to Preparation Instructions, steps 6, 7, 8, and 10.

1. Keep kit reagents on ice during reagent preparation steps. Equilibrate plate to room temperature before opening the sealed pouch.

2. Assay Diluent B (Item E) should be diluted 5-fold with deionized or distilled water.

3. Briefly centrifuge the Anti-ZAG Antibody vial (Item N) before use. Add 50 µL of 1x Assay Diluent B into the vial to prepare a detection antibody concentrate. Pipette up and down to mix gently.

4. The antibody concentrate should then be diluted 100-fold with 1x Assay Diluent B. This is the anti-ZAG antibody working solution, which will be used in Procedure, step 2.

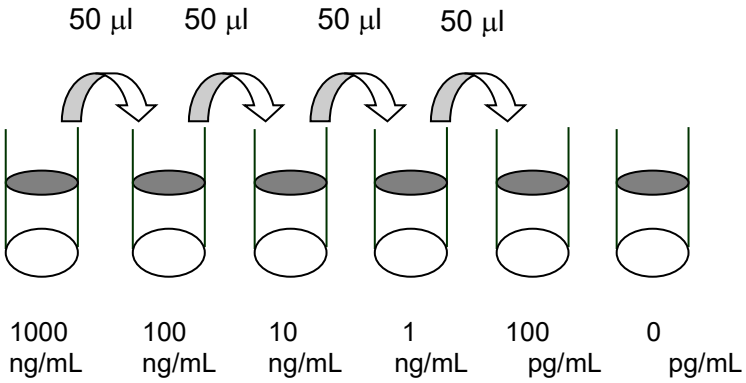
Note: The following steps may be done during the antibody incubation procedure (Procedure, step 2).

5. Briefly centrifuge the vial of Biotinylated ZAG (Item F) before use. Add 5 µl of Item F to 5 mL of the appropriate Assay Diluent. Pipette up and down to mix gently. The final concentration of biotinylated ZAG will be 50 ng/mL. This solution will only be used as the diluent in Preparation, step 6.

6. Preparation of Standards: Label 6 microtubes with the following concentrations: 1,000 ng/mL, 100 ng/mL, 10 ng/mL, 1 ng/mL, 100 pg/mL and 0 pg/mL. Pipette 450 µL of biotinylated ZAG solution into each tube, except for the 1,000 ng/mL (leave this one empty). It is very important to make sure the concentration of biotinylated ZAG is 50 ng/mL in all standards.

   a. Briefly centrifuge the vial of ZAG (Item C). In the tube labeled 1,000 ng/mL, pipette 8 µL of Item C and 792 µL of 50 ng/mL biotinylated ZAG solution (prepared in step 5). This is the ZAG stock solution (1,000 ng/mL ZAG, 50 ng/mL biotinylated ZAG). Mix thoroughly. This solution serves as the first standard.
   b. To make the 100 ng/mL standard, pipette 50 µL of ZAG stock solution the tube labeled 100 ng/mL. Mix thoroughly.
   c. Repeat this step with each successive concentration, preparing a dilution series as shown in Figure 1. Each time, use 450 µL of biotinylated ZAG and 50 µL of the prior concentration until 100 pg/mL is reached. Mix each tube thoroughly before the next transfer.
   d. The final tube (0 pg/mL ZAG, 50 ng/mL biotinylated ZAG) serves as the zero standard (or total binding).

Figure 1.
Dilution Series for Standards



7. Prepare a 10-fold dilution of Item F. To do this, add 2 µL of Item F to 18 µL of the appropriate Assay Diluent. This solution will be used in steps 8 and 10.

8. Positive Control Preparation: briefly centrifuge the positive control vial (Item M). To the tube of Item M, add 101 µL of 1x Assay Diluent B. Also add 2 µL of 10-fold diluted Item F (prepared in step 7) to the tube. This is a 2-fold dilution of the positive control. Mix thoroughly. The positive control is a cell culture medium sample with an expected signal between 10–30% of total binding (70–90% competition) if diluted as described. It may be diluted further if desired, but be sure the final concentration of biotinylated ZAG is 50 ng/mL.

9. If Item B (20x Wash Concentrate) contains visible crystals, warm to room temperature and mix gently until dissolved. Dilute 20 mL of Wash Buffer Concentrate into deionized or distilled water to yield 400 mL of 1x Wash Buffer.

10. Sample Preparation: Use Assay Diluent A plus biotinylated ZAG to dilute serum/plasma samples. For cell culture medium and other sample types, use 1x Assay Diluent B plus biotinylated ZAG as the diluent.

    Note: It is very important to make sure the final concentration of the biotinylated ZAG is 50 ng/mL in every sample. For example, to make a 4-fold dilution of sample, mix together 2.5 µL of 10-fold diluted Item F (prepared in step 7), 185 µL of appropriate Assay Diluent, and 62.5 µL of the sample; mix gently. The total volume is 250 µL, enough for duplicate wells on the microplate.

    Do not use Item F diluent from step 5 for sample preparation.

    If undiluted samples are used, biotinylated ZAG must still be added to a final concentration of 50 ng/mL. For example, Add 2.5 µL of 10-fold diluted Item F to 247.5 µL of sample.

11. Briefly centrifuge the HRP-Streptavidin vial (Item G) before use. The HRP-Streptavidin concentrate should be diluted 100-fold with 1x Assay Diluent B.

    Note: Do not use Assay Diluent A for HRP-Streptavidin preparation in step 11.

Storage/Stability

Store the kit at –20 °C. It remains active for up to 1 year. Avoid repeated freeze-thaw cycles.

The reconstituted standard should be stored at –20 °C or –70 °C (–70 °C is recommended). Opened microplate strips or reagents may be store for up to 1 month at 2–8 °C. Return unused wells to the pouch containing desiccant pack and reseal along entire edge.

Procedure

1. Keep kit reagents on ice during reagent preparation steps. It is recommended that all standards and samples be run at least in duplicate.

2. Add 100 µL of anti-ZAG antibody (see Preparation, step 4) to each well. Incubate for 1.5 hours at room temperature with gentle shaking (1–2 cycles/sec) or incubate overnight at 4 °C.

3. Discard the solution and wash wells 4 times with 1x Wash Buffer (200–300 µL each), Washing may be done with a multichannel pipette or an automated plate washer. Complete removal of liquid at each step is essential to good assay performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.

4. Add 100 µL of each standard (see Preparation, step 6), positive control (see Preparation, step 8) and sample (see Preparation, step 10) into appropriate wells. Be sure to include a blank well (Assay Diluent only). Cover wells and incubate for 2.5 hours at room temperature with gentle shaking (1–2 cycles/sec) or incubate overnight at 4 °C.

5. Discard the solution and wash 4 times as directed in step 3.

6. Add 100 µL of prepared HRP-Streptavidin solution (see Preparation, step 11) to each well. Incubate for 45 minutes with gentle shaking at room temperature. It is recommended that incubation time should not be shorter or longer than 45 minutes.

7. Discard the solution and wash 4 times as directed in step 3.

8. Add 100 µL of TMB One-Step Substrate Reagent (Item H) to each well. Incubate for 30 minutes at room temperature in the dark with gentle shaking (1–2 cycles/sec).

9. Add 50 µL of Stop Solution (Item I) to each well. Read absorbances at 450 nm immediately.

Results

Calculations

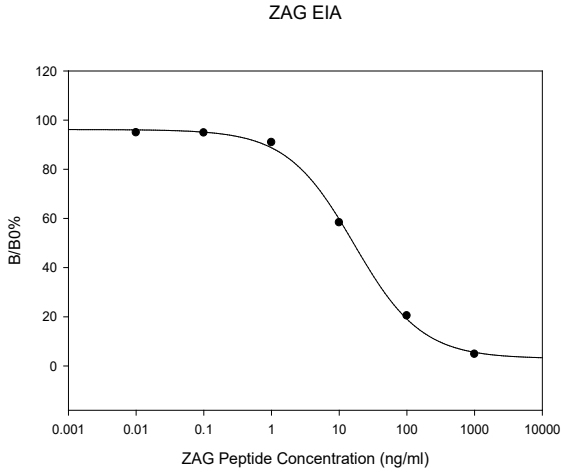
Calculate the mean absorbance for each set of duplicate standards, controls, and samples, and subtract the average zero standard optical density. Plot the standard curve on log-log graph paper or using Sigma plot software, with standard concentration on the x-axis and absorbance on the y-axis. Draw the best-fit straight line through the standard points.

$$\% \text{ absorbance} = (B - \text{blank OD})/(B_o - \text{blank OD})$$

where
B = OD of sample or standard and
B_o = OD of zero standard (total binding)

Typical Data

Standard curve(s) is for demonstration only. Standard curve(s) must be run with each assay.



Product Profile

Sensitivity: The minimum detectable dose of ZAG is 21 pg/mL.

Reproducibility:
Intra-Assay: CV <10%
Inter-Assay: CV <15%

Detection Range:
0.1–1,000 ng/mL

Specificity

This EIA kit recognizes human, mouse, and rat zinc-binding α-2 glycoprotein (ZAG)

Cross Reactivity: This EIA kit shows no cross-reactivity with any of the cytokines tested: Ghrelin, Nesfatin, NPY, and APC.

References

1. Marrades, M.P. et al., ZAG, a lipid mobilizing adipokine, is downregulated in human obesity. J. Physiol. Biochem., **64**(1), 61-66 (2008).

Appendix

Troubleshooting Guide

Problem	Cause	Solution
Poor standard curve	Inaccurate pipetting	Check pipettes
	Improper standard dilution	Ensure a brief spin of Item C and dissolve the powder thoroughly with gentle mixing.
Low signal	Too brief incubation times	Ensure sufficient incubation time; Procedure, step 2 may change to over night
	Inadequate reagent volumes or improper dilution	Check pipettes and ensure correct preparation
Large CV	Inaccurate pipetting	Check pipettes
High background	Plate is insufficiently washed	Review the manual for proper wash. If using a plate washer, check that all ports are unobstructed.
	Contaminated wash buffer	Make fresh wash buffer
Low sensitivity	Improper storage of the ELISA kit	Store the standard at <–20 °C after reconstitution, others at 4 °C. Keep substrate solution protected from light
	Stop solution	Stop solution should be added to each well before measurement.

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