

Human/Mouse/Rat AZGP1 / ZAG ELISA (Competitive EIA)

User Manual

Catalog No. LS-F15

It is important that you read this entire manual carefully before starting your experiment.

| Assay Specifications | 1 |
|-------------------------------------|----|
| Assay Principle | 2 |
| Kit Components | 4 |
| Kit Storage Other Required Supplies | 4 |
| Other Required Supplies | 4 |
| Assay Planning | 5 |
| Sample Collection | 6 |
| Sample Collection Notes | 8 |
| Reagent Preparation | 9 |
| Positive Control Preparation | 11 |
| Sample Preparation | 12 |
| Standard Preparation | 13 |
| Reagent Preparation Notes | 14 |
| Assay Procedure | 15 |
| Assay Procedure Notes | 16 |
| Assay Procedure Summary | 18 |
| Calculation of Results | 19 |
| Troubleshooting Guide | 20 |
| Assay Usage and Support | 22 |
| Returns, Refunds, Cancellations | 23 |

ASSAY SPECIFICATIONS

Target: AZGP1 / ZAG

Synonyms: AZGP1 / ZAG alpha-2-glycoprotein 1, zinc-binding

AZGP1-ZAG AZGP1 Alpha-2-glycoprotein 1, zinc Alpha-2-glycoprotein, zinc ZA2G Zn-alpha-2glycoprotein Zn-alpha2-glycoprotein ZNGP1 AZGP1

ZAG Zn-alpha-2-GP Zinc-alpha-2-glycoprotein

Specificity: Recombinant and natural Mouse/Human/Rat AZGP1

/ ZAG 278aa. No other isoforms have been reported. This kit detects AZGP1 / ZAG. This ELISA kit shows no cross-reactivity with any of the adipokines tested: Ghrelin, Nesfatin, NPY and APC. This claim is limited by existing techniques therefore cross-reactivity may

exist with untested analogs.

Sample Types: This kit is intended for use with samples such as

human, mouse and rat serum and cell culture supernatants. It has been empirically tested using

the standard supplied with the kit (typically a

recombinant protein).

Detection Range: 0.1-1,000 ng/ml

Sensitivity: Typically less than 0.1 ng/ml

Performance: Intra-Assay CV (<10%); Inter-Assay CV (<15%)

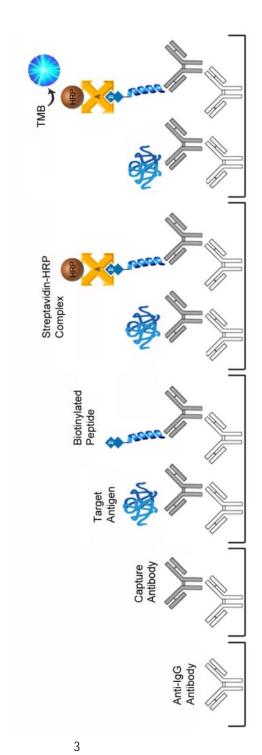
Limitations: This kit is for **Research Use Only** and is not intended

for diagnostic use. This kit is not approved for use in

humans or for clinical diagnosis.

ASSAY PRINCIPLE

This assay is based on the Competitive EIA principle. Each well of the supplied microtiter plate has been pre-coated with an anti-rabbit antibody. An antibody specific to the target antigen (the capture antibody) is then added to each well and binds to the anti-rabbit antibody. Next a biotinylated synthetic antigen peptide is added to each well along with either the non-biotinylated standard peptide or the test sample. The biotinylated synthetic antigen peptide competes with the non-biotinylated standard peptide or native antigen in the sample to bind with the capture antibody. Unbound antigen and peptide is washed away. An Avidin-Horseradish Peroxidase (HRP) conjugate is then added which binds to the biotin. Unbound HRP-conjugate is washed away. A TMB substrate is then added which reacts with the HRP enzyme resulting in color development. A sulfuric acid stop solution is added to terminate color development reaction and then the optical density (OD) of the well is measured at a wavelength of 450 nm ± 2 nm. The OD of an unknown sample can then be compared to an OD standard curve generated using known antigen concentrations in order to determine its antigen concentration. In contract to typical Sandwich ELISA assays, in competition assays the greater the amount of antigen in the sample, the lower the color development and optical density reading.



ASSAY PRINCIPLE IMAGE

KIT COMPONENTS

| Component | Quantity |
|----------------------------------|-----------------|
| Coated 96-well Strip Plate | 1 |
| Standard | 2 vials |
| Assay Diluent A* | 1 vial x 30ml |
| Assay Diluent B (5x)* | 1 vial x 15ml |
| Capture Antibody | 2 vials |
| Biotinylated Peptide | 2 vials |
| HRP-Streptavidin Conjugate (50x) | 1 vial x 600 μl |
| Positive Control | 1 vial |
| Wash Buffer (20x) | 1 vial x 25 ml |
| TMB Substrate | 1 vial x 12 ml |
| Stop Solution | 1 vial x 8 ml |
| Adhesive Plate Sealers | 4 |
| Instruction Manual | 1 |

^{*}May contain 0.09% sodium azide

KIT STORAGE

The Standard, Biotinylated Peptide, and Positive Control should be stored at -20°C/-80°C upon arrival. Avoid freeze thaw cycles. Store all other kit components at -20°C. Once individual reagents are opened it is recommended that the kit be used within 1 month. Unused Strip Plate wells should be stored at 2°-8°C for 1 month in a sealed bag containing desiccant in order to minimize exposure to moisture. Do not use the kit beyond its expiration date.

OTHER REQUIRED SUPPLIES

- Microplate reader with 450nm wavelength filter
- High-precision pipette and sterile pipette tips
- Eppendorf tubes
- 37°C incubator
- Deionized or distilled water
- Absorbent paper

ASSAY PLANNING

Before using this kit, researchers should consider the following:

- 1. Read this manual in its entirety in order to minimize the chance of error.
- 2. Confirm that you have the appropriate non-supplied equipment available.
- 3. Confirm that the species, target antigen, and sensitivity of this kit are appropriate for your intended application.
- 4. Confirm that your samples have been prepared appropriately based upon recommendations (see Sample Preparation) and that you have sufficient sample volume for use in the assay.
- 5. When first using a kit, appropriate validation steps should be taken before using valuable samples. Confirm that the kit adequately detects the target antigen in your intended sample type(s) by running control samples.
- 6. If the concentration of target antigen within your samples is unknown, a preliminary experiment should be run using a control sample to determine the optimal sample dilution (see Experimental Layout and Sample Preparation).
- 7. Ensure that the kit is properly stored and do not use it beyond its expiration date.
- 8. When using multiple lots of the same kit do not substitute reagents from one kit to another. Review each manual carefully as changes can occur between lots. To control for inter-assay variability include a carry-over control sample.

SAMPLE COLLECTION

This assay is recommended for use with Mouse/Human/Rat serum and cell culture supernatants. The sample collection protocols below have been provided for your reference.

Breast Milk - Centrifuge samples for 20 minutes at 1000×g to remove particulates. Collect the supernatant for assaying.

Cell Lysates - Collect and pellet the cells by centrifugation and remove the supernatant. Wash the cells 3 times with PBS* then resuspend in PBS*. Lyse the cells by ultrasonication 4 times. Alternatively freeze the cells to -20°C and thaw to room temperature 3 times. Centrifuge at 1500×g for 10 minutes at 2 - 8°C to remove cellular debris. Collect the supernatant for assaying.

Erythrocyte Lysates - Centrifuge whole blood for 20 minutes at 1000×g to pellet the cells and remove the supernatant. Wash the cells 3 times with PBS* then resuspend in PBS*. Freeze (-20°C)/thaw (room temperature) the cells 3 times. Centrifuge at 5,000×g for 10 minutes at 2-8°C to remove cellular debris. Collect the supernatant for assaying. Erythrocyte lysates must be diluted with Sample Diluent before running.

Plasma - Collect plasma using Heparin, EDTA, or Citrate as an anticoagulant. Centrifuge samples for 15 minutes at 1000×g at 2–8°C within 30 minutes of collection. Collect the supernatant for assaying.

Platelet-Poor Plasma - Collect plasma using Heparin, EDTA, or Citrate as an anticoagulant. **Centrifuge samples for 15 minutes at 1000×g at 2–8°C** within 30 minutes of collection. It is recommended that samples should be centrifuged for 10 minutes at 10,000×g for complete platelet removal. Collect the supernatant for assaying.

Sperm and Seminal Plasma – Allow semen to liquefy at room temperature or 37°C. After liquefaction, centrifuge at 2,000×g for 10-15 minutes. Collect seminal plasma supernatant for assaying. Wash the precipitated protein 3 times with PBS* then resuspend in PBS*. Lyse the cells by ultrasonication then centrifuge at 2,000×g for 10-15 minutes. Collect the supernatant for assaying.

Serum - Use a serum separator tube and allow samples to clot for 2 hours at room temperature or overnight at 4°C before centrifugation for 20 minutes at approximately 1000×g. Collect the supernatant for assaying.

Tissue Homogenates – Because preparation methods for tissue homogenates vary depending upon tissue type, users should research tissue specific conditions independently. The following is one example only. Rinse tissues in PBS* to remove excess blood and weigh before homogenization. Finely mince tissues and homogenize them in 5-10mL of PBS*with a glass homogenizer on ice. Lyse the cells by ultrasonication or freeze (-20°C)/thaw (room temperature) 3 times. Centrifuge homogenate at 5000×g for 5 minutes. Collect the supernatant for assaying.

Urine - Aseptically collect the first urine of the day (mid-stream), voided directly into a sterile container. Centrifuge to remove particulate matter and collect the supernatant for assaying.

Cell culture supernatants, cerebrospinal, follicular, and lung lavage fluids, saliva, sweat, tears, and other biological fluids - Centrifuge samples for 20 minutes at 1000×g to remove particulates. Collect the supernatant for assaying.

* 1xPBS (0.02mol/L pH7.0-7.2)

SAMPLE COLLECTION NOTES

- LSBio recommends that samples are used immediately upon preparation. Alternatively, samples stored at 2-8°C should be used within 5 days. For long-term storage sample aliquots should be prepared and stored at -20°C if used within 1 month, or -80°C if used within 6 months. Long term storage can result in protein degradation and denaturation, which may result in inaccurate results.
- 2. Avoid repeated freeze/thaw cycles for all samples.
- 3. In the event that a sample type not listed above is intended to be used with the kit, it is recommended that the customer conduct validation experiments in order to be confident in the results.
- 4. Due to chemical interference, the use of tissue or cell extraction samples prepared by chemical lysis buffers may result in inaccurate results.
- Due to factors including cell viability, cell number, or sampling time, samples from cell culture supernatant may not be detected by the kit.
- 6. Samples should be brought to room temperature (18-25°C) before performing the assay without the use of extra heating.
- Sample concentrations should be predicted before being used in the assay. If the sample concentration is not within the range of the standard curve, users must determine the optimal sample dilutions for their particular experiments.
- 8. LSBio is responsible for the quality and performance of the kit components but is NOT responsible for the performance of customer supplied samples used with the kit.

REAGENT PREPARATION

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

1x Wash Buffer (for use in Assay Procedure (page 15)): If crystals have formed in the concentrate, warm to room temperature and mix it gently until crystals have completely dissolved. Prepare 400 ml of Working Wash Buffer by diluting the supplied 20 ml of 20x Wash Buffer Concentrate with 380 ml of deionized or distilled water. Wash Buffer can be stored at 4°C once prepared.

1x Assay Diluent B: Prepare 75 ml of Assay Diluent B by diluting the supplied 15 ml of 5x Assay Diluent Concentrate with 60 ml of deionized or distilled water. Assay Diluent can be stored at 4°C once prepared.

100x Capture Antibody Concentrate: Briefly spin down the Capture Antibody and add 50 μ l of 1x Assay Diluent. The Capture Antibody Concentrate can be stored at 4°C for 5 days.

1x Capture Antibody Solution: (for use in Assay Procedure (page 15)): Calculate the volume of Capture Antibody Solution needed for your particular experiment and prepare that volume by diluting the Capture Antibody Concentrate 100-fold (1:100) with 1x Assay Diluent (use **Assay Diluent A** if your samples are serum or plasma, and **Assay Diluent B** for all other sample types). Capture Antibody Solution must be prepared fresh for each experiment and cannot be stored.

Working Biotinylated Peptide Solution (100 ng/ml): (for use in Control, Sample, and Standard preparation (pages 11, 12, 13) Briefly spin down the Biotinylated Peptide and combine the entire volume of the vial with 10ml of 1x Assay Diluent (use Assay Diluent A if your samples are serum or plasma, and Assay Diluent B for all other sample types) and mixing gently. The final concentration of the Biotinylated Peptide in this solution is 100 ng/ml.

HRP-Streptavidin Working Solution (for use in Assay Procedure (page 15)): Gently mix the stock solution before use. Calculate the volume of HRP-Streptavidin Working Solution needed for your particular experiment and prepare that volume by diluting the HRP-Streptavidin Conjugate Solution 50-fold (1:50) with 1x Assay Diluent B. HRP-Streptavidin working solution must be prepared fresh for each experiment and cannot be stored.

TMB Substrate (for use in Assay Procedure (page 15)): Using sterile techniques remove the needed volume of TMB Substrate Solution for the number of wells you are planning to run. Dispose of unused TMB Substrate Solution rather than returning it to the stock container.



POSITIVE CONTROL PREPARATION

The Positive Control is a cell culture medium sample that is meant to be a system control to verify that the detection and kit components are working. The resulting OD will not be used in any calculations.

Briefly spin down the Positive Control before opening. Combine 100 μ l of Positive Control with 100 μ l of Working Biotinylated Peptide Solution (100 ng/ml) and mix thoroughly. This is a 2-fold dilution of the Biotinylated Peptide and its final concertation in this solution is 50 ng/ml.

If this positive control does not produce a positive signal in the assay please contact LifeSpan Technical Support.

The positive control may be diluted further if desired, but be sure that the final concentration of Biotinylated Peptide is 50 ng/ml.

SAMPLE PREPARATION

<u>Please predict the concentration of your samples before assaying.</u> The resulting optical density (OD) values of your sample must fall within the OD readings of the standard curve in order for the calculated antigen concentration to be accurate. A dilution series of each sample may be necessary. The following dilutions will provide you with a 2-fold to 100-fold range.

| Dilution | Sample | 1x Assay | Working Biotinylated | Final |
|----------|--------|----------|----------------------|-----------------|
| | | Diluent* | Peptide Solution | Volume |
| | | | (100 ng/ml) | |
| 2-fold | 125 µl | 0 μΙ | 125 μΙ | 250 μΙ |
| 4-fold | 65 µl | 65 µl | 130 μΙ | 260 μΙ |
| 10-fold | 25 µl | 100 μΙ | 125 μΙ | 250 μΙ |
| 100-fold | 10 μΙ | 490 µl | 500 μl | 100 0 μΙ |

^{*} Use **Assay Diluent A** for serum or plasma, and **Assay Diluent B** for all other sample types.

It is recommended that all samples are run in duplicate (100 µl/well).

When samples are added to the wells, they must contain Biotinylated Peptide at a concertation of **50 ng/ml**.

Special Cases:

In this assay human serum typically requires an 8-fold dilution, mouse serum typically requires a 2-fold dilution and rat serum typically requires a 10-fold dilution in order for their OD readings to fall within the Standard Curve of this assay. However, due to sample variation, it is recommended that researchers determine the optimal dilution for their samples as described above.

STANDARD PREPARATION

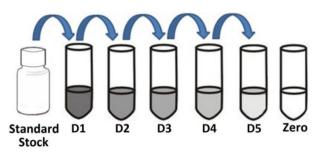
The following are instructions for the preparation of a Standard dilution series which will be used to generate the standard curve. The standard curve is then used to determine the concentration of target antigen in unknown samples (see the **Calculation of Results** section). The following will prepare sufficient volumes to run the Standard dilution series in duplicate. Reconstituted Standard and prepared standard dilutions should be used immediately and not stored for future use.

50 ng/ml Biotinylated Peptide Solution: Combine 2.0 ml of Working Biotinylated Peptide Solution (100 ng/ml) with 2.0 ml of 1x Assay Diluent (use **Assay Diluent A** if your samples are serum or plasma, and **Assay Diluent B** for all other sample types) and mix thoroughly.

Standard Stock Solution: Briefly spin down the Standard prior to opening.

Preparation of Standard Dilutions: Prepare the following 10-fold serial dilutions.

| D1 (1,000 ng/ml): | Combine 8 µl of the Standard Stock Solution with 792 µl of 50 ng/ml Biotinylated Peptide Solution. |
|--------------------------|----------------------------------------------------------------------------------------------------|
| D2 (100 ng/ml): | Pipette 50μl of D1 into 450μl of 50 ng/ml Biotinylated Peptide Solution |
| D3 (10 ng/ml): | Pipette 50μl of D2 into 450μl of 50 ng/ml Biotinylated Peptide Solution |
| D4 (1 ng/ml): | Pipette 50μl of D3 into 450μl of 50 ng/ml Biotinylated Peptide Solution |
| D5 (0.1 ng/ml): | Pipette 50μl of D4 into 450μl of 50 ng/ml Biotinylated Peptide Solution |
| Blank (0 ng/ml): | Use 500μl of 50 ng/ml Biotinylated Peptide Solution alone. |



REAGENT PREPARATION NOTES

- 1. It is highly recommended that standard curves and samples are run in duplicate within each experiment.
- Once resuspended, standards should be used immediately, and used only once. Long-term storage of reconstituted standards is NOT recommended.
- 3. All solutions prepared from concentrates are intended for one-time use. Do not reuse solutions.
- 4. Do not prepare Standard dilutions directly in wells.
- 5. Prepared Reagents may adhere to the tube wall or cap during transport; centrifuge tubes briefly before opening.
- 6. All solutions should be gently mixed prior to use.
- 7. Reconstitute stock reagents in strict accordance with the instructions provided.
- 8. To minimize imprecision caused by pipetting, ensure that pipettes are calibrated. Pipetting volumes of less than $10\mu L$ is not recommended.
- Substrate Solution is easily contaminated; sterility precautions should be taken. Substrate Solution should also be protected from light.
- 10. Do not substitute reagents from one kit lot to another. Use only those reagents supplied within this kit.
- 11. Due to the antigen specificity of the antibodies used in this assay, native or recombinant proteins from other manufacturers may not be detected by this kit.

ASSAY PROCEDURE

Keep kit reagents on ice during reagent preparation steps. Prepare all reagents, working standards, and samples as directed in the previous sections.

- 1. Add 100 μl of **1x Capture Antibody Solution** to each well, cover with a plate sealer, and incubate for 1.5 hours at room temperature, or overnight at 4°C, with gentle shaking (1-2 cycles/sec).
- Aspirate the liquid from each well and wash 4 times. Wash by adding approximately 200-300 μl of Wash Buffer using a squirt bottle, multi-channel pipette, manifold dispenser or automated washer. After the last wash, aspirate to remove any remaining Wash Buffer then invert the plate and tap against clean absorbent paper.
- 3. Add 100 μ l of **Standard, Blank, Positive Control, or Sample** per well, cover with a plate sealer, and incubate for 2.5 hours at room temperature, or overnight at 4°C, with gentle shaking (1-2 cycles/sec).
- 4. Aspirate and wash the wells 4 times as outlined in step 2.
- 5. Add 100 μ l of **HRP-Streptavidin Working Solution** to each well and incubate for 45 minutes at room temperature with gentle shaking (1-2 cycles/sec).
- 6. Aspirate and wash the wells 4 times as outlined in step 2.
- Add 100 µl of **TMB Substrate** to each well and incubate for 30 minutes at room temperature in the dark with gentle shaking (1-2 cycles/sec). Monitor periodically until optimal color development has been achieved.
- 8. Add 50 µl of **Stop Solution** to each well and record the total development time. The blue color will change to yellow. If color change does not appear uniform, gently tap the plate to ensure thorough mixing. The Stop Solution should be added to wells in the same order and timing as the substrate solution.
- 9. Determine the optical density (OD value) of each well immediately using a microplate reader set to 450 nm.

ASSAY PROCEDURE NOTES

- 1. **ELISA Plate**: Keep appropriate numbers of strips for 1 experiment and remove extra strips from microtiter plate. Removed strips should be placed in a sealed bag containing desiccant and stored at -20°C.
- 2. **Solutions**: To avoid cross-contamination, change pipette tips between additions of each standard, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.
- Applying Solutions: All solutions should be added to the bottom of the ELISA plate well. Avoid touching the inside wall of the well. Avoid foaming when possible.
- 4. **Assay Timing**: The interval between adding sample to the first and last wells should be minimized. Delays will increase the incubation time differential between wells, which will significantly affect the experimental accuracy and repeatability. For each step in the procedure, total dispensing time for addition of reagents or samples should not exceed 10 minutes.
- 5. **Incubation**: To prevent evaporation and ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary. Do not allow wells to sit uncovered for extended periods of time between incubation steps. Do not let wells dry out at any time during the assay. Strictly observe the recommended incubation times and temperatures.
- 6. **Washing**: Proper washing procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings. Residual liquid in the reaction wells should be patted dry against absorbent paper during the washing process. Do not put absorbent paper directly into the reaction wells.
- Controlling Substrate Reaction Time: After the addition of the TMB Substrate, periodically monitor the color development. Stop color development before the color becomes too deep by adding Stop Solution. Excessively strong color will result in inaccurate absorbance readings.
- 8. **Reading**: The microplate reader should be preheated and programmed prior to use. Prior to taking OD readings, remove any

- residual liquid or fingerprints from the underside of the plate and confirm that there are no bubbles in the wells.
- 9. **Reaction Time Control**: Control reaction time should be strictly followed as outlined.
- 10. **Stop Solution**: The Stop Solution contains an acid, therefore proper precautions should be taken during its use, such as protection of the eyes, hands, face, and clothing.
- 11. **Mixing**: During incubation times, the use of a micro-oscillator at low frequency is recommended. Sufficient and gentle mixing is particularly important in producing reliable results.
- 12. To minimize external influence on the assay performance, operational procedures and lab conditions (such as room temperature, humidity, incubator temperature) should be strictly controlled. It is also strongly suggested that the whole assay is performed by the same operator from the beginning to the end.

ASSAY PROCEDURE SUMMARY

Prepare all reagents, samples and standards.

Add 100 µl of **Capture Antibody** to each well and incubate for 1.5 hours at room temperature or overnight at 4°C.

Aspirate and wash 4 times.

Add 100 µl of **Standard**, **Blank**, **Positive Control**, or **Sample** to each well and incubate for 2.5 hours at room temperature or overnight at 4°C.

Aspirate and wash 4 times.

Add 100 µl of **HRP-Streptavidin Solution** and incubate for 45 minutes at room temperature.

Aspirate and wash 4 times.

Add 100 μ l of **TMB Substrate** and incubate for 30 minutes at room temperature.

Add 50 µl of Stop Solution.

Read immediately at 450nm.

CALCULATION OF RESULTS

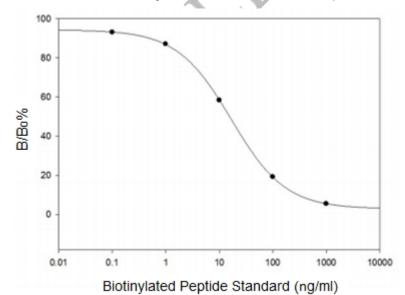
Calculate the mean absorbance for each set of duplicate stands, controls, and samples and subtract the blank optical density. Plot the standard curve using SigmaPlot software (or other software which can perform four-parameter logistic regression models), with standard concentration on the x-axis and percentage of absorbance (see calculation below) on the y-axis. Draw the best-fit curve through the standard points.

Percentage absorbance = $(B - blank OD)/(B_0 - blank OD)$ where

B = OD of sample or standard and

 $B_0 = OD$ of zero standard (total binding)

Typical Data: The following standard curve is an example only and should not be used to calculate results for tested samples. A new standard curve must be generated for each set of samples tested.



TROUBLESHOOTING GUIDE

| Problem | Possible Cause | Solution | |
|--------------------------|--------------------------------------|---------------------------------------------------------------------------------------------------|--|
| Poor standard curve | Inaccurate pipetting | Check pipettes | |
| | Improper standard dilution | Briefly spin the vial of standard and dissolve the powder thoroughly by a gentle mix. | |
| | Wells not completely aspirated | Completely aspirate wells between steps. | |
| Low signal | Too brief incubation times | Ensure sufficient incubation time. | |
| | Incorrect assay temperature | Use recommended incubation temperature. Bring substrate to room temperature before use. | |
| | Inadequate reagent volumes | Check pipettes and ensure correct preparation. | |
| | Improper dilution | | |
| Deep color but low value | Plate reader settings not optimal | Verify the wavelength and filter setting in the plate reader. | |
| | | Turn on and warm-up the plate reader prior to use. | |

Troubleshooting Guide (continued)

| Problem | Possible Cause | Solution |
|-----------------|--------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------|
| Large CV | Inaccurate pipetting | Check pipettes |
| High background | Concentration of detector too high | Use recommended dilution factor. |
| | Plate is insufficiently washed | Review the manual for proper washing instructions. 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 | All the reagents should be stored according to the instructions. |
| | Stop solution not added | Stop solution should be added to each well before measurement. |

ASSAY USAGE AND SUPPORT

This kit is for **Research Use Only** and is not intended for diagnostic use. This kit is not approved for use in humans or for clinical diagnosis. This kit should not be used beyond the expiration date printed on the lot specific kit label.

Warning: This reagent may contain sodium azide and sulfuric acid. The chemical, physical, and toxicological properties of these materials have not been thoroughly investigated. Standard Laboratory Practices should be followed. Avoid skin and eye contact, inhalation, and ingestion. Sodium azide forms hydrazoic acid under acidic conditions and may react with lead or copper plumbing to form highly explosive metal azides. On disposal, flush with large volumes of water to prevent accumulation.

The LifeSpan Guarantee: LifeSpan guarantees the integrity of all components contained with an immunoassay kit, and that the standards provided will produce a standard curve sufficient for the quantification of target antigen concentrations that fall within the specified range of the kit. Due to the variable nature of sample types and preparations, LifeSpan cannot guarantee that the target antigen will be detectable in customer-supplied samples. For this reason, LifeSpan strongly recommends that customers conduct validation experiments, using positive control samples generated in a similar manner to the experimental samples, before using valuable research specimens. Due to the perishable nature of ELISA kits, orders of greater than 5 units of a single catalog number cannot be returned upon shipment, and are not eligible for refund.

Technical Support: LifeSpan's knowledgeable staff scientists are available to answer any questions about this kit. Email your detailed questions to Technical.Support@LSBio.com.

RETURNS, REFUNDS, CANCELLATIONS

Any problems with LifeSpan products must be reported to LifeSpan within 10 days of product receipt. The customer must obtain written authorization from LifeSpan before returning items. To request that goods be returned, please contact LifeSpan Technical Support. If an error by LifeSpan Biosciences results in shipment of an incorrect order, LifeSpan will, at its option, either ship a replacement order at no charge, or credit the customer's account for the original product shipped in error. Returns and cancellations may be subject to a 30% restocking fee. Conditions & Warranty: All LifeSpan products are intended for Research Use Only and are not for use in human therapeutic or diagnostic applications. The information supplied with each product is believed to be accurate, but no warranty or quarantee is offered for the products, because the ultimate conditions of use are beyond LifeSpan's control. The information supplied with each product is not to be construed as a recommendation to use this product in violation of any patent, and LifeSpan will not be held responsible for any infringement or other violation that may occur with the use of its products. Under no event will LifeSpan be responsible for any loss of profit or indirect consequential damage, including, but not limited to, personal injuries resulting from use of these products. LifeSpan's liability to any user of Products for damages that do not result from any fault of the user, will be limited to replacement of the Product(s) only, and in no event shall LifeSpan's liability exceed the actual price received by LifeSpan for the Product(s) at issue. LifeSpan shall not be liable for any indirect, special, incidental or consequential damages, LIFESPAN FURTHER DISCLAIMS ANY AND ALL EXPRESS AND IMPLIED OR STATUTORY WARRANTIES WITH RESPECT TO THE PRODUCTS, INCLUDING BUT NOT LIMITED TO ANY IMPLIED. WARRANTIES OF MERCHANTABILITY OR FITNESS FOR A PARTICULAR PURPOSE. LifeSpan disclaims any and all responsibility for any injury or damage which may be caused by the fault of the user.

For Research Use Only. Not approved for use in humans or for clinical diagnosis.



2 Shaker Rd Suite B101 Shirley, MA 01464 Tel: 206.374.1102 Fax: 206.577.4565 Technical.Support@LSBio.com