

# **LSBio™ Mouse Anti-GAD autoantibody (IgM) Direct ELISA Kit**

**Catalog No. LS-F10309**

## **User Manual**

**Please Read the Manual Carefully  
Before Starting your Experiment**



For research use only. Not approved for use in humans or for clinical diagnosis

## **PRINCIPLE OF THE ASSAY**

This assay employs the qualitative enzyme immunoassay technique. The microtiter plate provided in this kit has been pre-coated with antigen. Samples are pipetted into the wells with anti-mouse IgM conjugated Horseradish Peroxidase (HRP). Any antibodies specific for the antigen present will bind to the pre-coated antigen. Following a wash to remove any unbound reagent, a substrate solution is added to the wells and color develops in proportion to the amount of mouse GAD autoantibody(IgM) bound in the initial step. The color development is stopped and the intensity of the color is measured.

## **SPECIFICITY**

This assay has high sensitivity and excellent specificity for detection of mouse GAD autoantibody(IgM). No significant cross-reactivity or interference between mouse GAD autoantibody(IgM) and analogues was observed.

**Note:** Limited by current skills and knowledge, it is impossible for us to complete the cross-reactivity detection between mouse GAD autoantibody(IgM) and all the analogues, therefore, cross reaction may still exist.

## **PRECISION**

### **Intra-assay Precision (Precision within an assay): CV%<15%**

Three samples of known concentration were tested twenty times on one plate to assess.

### **Inter-assay Precision (Precision between assays): CV%<15%**

Three samples of known concentration were tested in twenty assays to assess.

## **LIMITATIONS OF THE PROCEDURE**

- **FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.**
- The kit should not be used beyond the expiration date on the kit label.
- Do not mix or substitute reagents with those from other lots or sources.
- Any variation in operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.
- This assay is designed to eliminate interference by soluble receptors, binding proteins, and other factors present in biological samples. Until all factors have been tested in the Immunoassay, the possibility of interference cannot be excluded.

## **MATERIALS PROVIDED**

Reagents	Quantity
Coated assay plate	1(96 wells)
Standard	3 x 1 ml
Negative Control	1 x 1 ml
Positive Control	1 x 1 ml
Sample Diluent	1 x 20 ml
HRP-conjugate	1 x 10 ml
Wash Buffer (25 x concentrate)	1 x 20 ml
Substrate A	1 x 5 ml
Substrate B	1 x 5 ml
Stop Solution	1 x 5 ml
Adhesive Strip (For 96 wells)	4
Instruction manual	1

## **STANDARD CONCENTRATION**

Tube	S1	S2	S3
Concentration	0.4	0.9	1.5

## **STORAGE**

Unopened kit	Store at 2 - 8°C. Do not use the kit beyond the expiration date.
Opened kit	May be stored for up to 1 month at 2 - 8° C.

**\*Provided this is within the expiration date of the kit.**

### **OTHER SUPPLIES REQUIRED**

- Microplate reader capable of measuring absorbance at 450 nm, with the correction wavelength set at 540 nm or 570 nm.
- An incubator which can provide stable incubation conditions up to 37°C±0.5°C.
- Squirt bottle, manifold dispenser, or automated microplate washer.  
Absorbent paper for blotting the microtiter plate.  
100ml and 500ml graduated cylinders.
- Deionized or distilled water.  
Pipettes and pipette tips.
- Test tubes for dilution.

### **PRECAUTIONS**

The Stop Solution provided with this kit is an acid solution. Wear eye, hand, face, and clothing protection when using this material.

**Note:**

1. Lifespan is only responsible for the kit itself, but not for the samples consumed during the assay. The user should calculate the possible amount of the samples used in the whole test. Please reserve sufficient samples in advance.
2. Samples to be used within 5 days may be stored at 2-8°C, otherwise samples must be stored at -20°C ( $\leq 1$ month) or -80°C ( $\leq 2$ month) to avoid loss of bioactivity and contamination.
3. Grossly hemolyzed samples are not suitable for use in this assay.
4. If the samples are not indicated in the manual, a preliminary experiment to determine the validity of the kit is necessary.
5. Please predict the concentration before assaying. If values for these are not within the range of the standard curve, users must determine the optimal sample dilutions for their particular experiments.
6. Tissue or cell extraction samples prepared by chemical lysis buffer may cause unexpected ELISA results due to the impacts of certain chemicals.
7. Owing to the possibility of mismatching between antigen from other resource and antibody used in our kits (e.g., antibody targets conformational epitope rather than linear epitope), some native or recombinant proteins from other manufacturers may not be recognized by our products.
8. Influenced by the factors including cell viability, cell number and also sampling time, samples from cell culture supernatant may not be detected by the kit.
9. Fresh samples without long time storage are recommended for the test. Otherwise, protein degradation and denaturalization may occur in those samples and finally lead to wrong results.

## **REAGENT PREPARATION**

### **Note:**

- **Kindly use graduated containers to prepare the reagent. Please don't prepare the reagent directly in the Diluent vials provided in the kit.**
- Bring all reagents to room temperature (18-25°C) before use for 30min.
- Distilled water is recommended to be used to make the preparation for reagents or samples. Contaminated water or container for reagent preparation will influence the detection result.

**Wash Buffer(1x)-** If crystals have formed in the concentrate, warm up to room temperature and mix gently until the crystals have completely dissolved. Dilute 20 ml of Wash Buffer Concentrate (25 x) into deionized or distilled water to prepare 500 ml of Wash Buffer (1 x).

## ASSAY PROCEDURE

**Bring all reagents and samples to room temperature before use. Centrifuge the sample again after thawing before the assay. It is recommended that all samples and controls be assayed in duplicate.**

1. Prepare all reagents, and samples as directed in the previous sections. 2. Refer to the Assay Layout Sheet to determine the number of wells to be used and put any remaining wells and the desiccant back into the pouch and seal the ziploc, store unused wells at 4°C.
  3. Set a **Blank** well without any solution.
  4. Add 100µl of **Negative Control, Positive Control, standard or diluted Sample** per well. Samples and controls must be assayed in duplicate. Cover with the adhesive strip provided. Incubate for 60 minutes at 37°C.
  5. Aspirate each well and wash, repeating the process four times for a total of five washes. Wash by filling each well with Wash Buffer (300µl) using a squirt bottle, multi-channel pipette, manifold dispenser, or autowasher, and let it stand for 30 seconds, complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
  6. Add 100µl of **HRP-conjugate** to each well (not to Blank!). Cover the microtiter plate with the adhesive strip. Incubate for 60 minutes at 37°C. Repeat the aspiration/wash process for five times as in step 5. 7.
  8. Add 50µl of **Substrate A** and 50µl **Substrate B** to each well. Incubate for 15 minutes at 37°C. **Protect from light.**
  9. Add 50µl of **Stop Solution** to each well, gently tap the plate to ensure thorough mixing.
- Take blank well as zero, determine the optical density of each well within 10. 10 minutes, using a microplate reader set to 450 nm.

**\*Samples may require dilution. Please refer to Sample Preparation section.**



Note:

1. The final experimental results will be closely related to validity of the products, operation skills of the end users and the experimental environments.
2. Samples or reagents addition: Please carefully add samples to wells and mix gently to avoid foaming. Do not touch the well wall as possible. For each step in the procedure, total dispensing time for addition of reagents or samples to the assay plate should not exceed 10 minutes. This will ensure equal elapsed time for each pipetting step, without interruption. Duplication of all specimens, although not required, is recommended. To avoid cross-contamination, change pipette tips between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.
3. Incubation: To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary. Do not allow wells to sit uncovered for extended periods between incubation steps. Once reagents have been added to the well strips, DO NOT let the strips DRY at any time during the assay. Incubation time and temperature must be observed.
4. Washing: The wash procedure is critical. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Solution by aspirating or decanting and remove any drop of water and fingerprint on the bottom of the plate. Insufficient washing will result in poor precision and falsely elevated absorbance reading. When using an automated plate washer, adding a 30 second soak period following the addition of wash buffer, and/or rotating the plate 180 degrees between wash steps may improve assay precision.
5. Controlling of reaction time: Observe the change of color after adding TMB Substrate (e.g. observation once every 10 minutes), TMB Substrate should change from colorless or light blue to gradations of blue. If the color is too deep, add Stop Solution in advance to avoid excessively strong reaction which will result in inaccurate absorbance reading.
6. TMB Substrate is easily contaminated. TMB Substrate should remain colorless or light blue until added to the plate. Please protect it from light.
7. Stop Solution should be added to the plate in the same order as the TMB Substrate. The color developed in the wells will turn from blue to yellow upon addition of the Stop Solution. Wells that are green in color indicate that the Stop Solution has not mixed thoroughly with the TMB Substrate.

## **CALCULATION OF RESULTS**

For calculation the valence of mouse GAD autoantibody(IgM), compare the sample well with control.

Create a standard curve by plotting the mean absorbance for each standard on the Y-axis against the concentration on the X-axis and draw a best fit curve through the points on the graph. Determine the concentration of mouse GAD autoantibody(IgM) after read the absorbance on the graph.

The negative control OD value should be less than 0.200, and the positive control OD value should be greater than 0.800. Or else, repeat the test.

- If the concentration of sample less than 1.00: Negative
- If the concentration of sample between 1.0-1.05: Gray Zone
- If the concentration of sample greater than 1.05: Positive

**Important Note:** During shipment, small volumes of product will occasionally become entrapped in the seal of the product vial. We recommend briefly centrifuging the vial to dislodge any liquid in the container's cap prior to opening.

**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.

**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 guarantee 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, 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.**



2401 Fourth Avenue Suite 900 Seattle, WA 98121

Tel: 206.374.1102

Fax: 206.577.4565

[Technical.Support@LSBio.com](mailto:Technical.Support@LSBio.com)