**(For scientific research use only, not for clinical diagnosis!)**

# Phytogibberellin 6 (GA6) ELISA

## Kit Instructions for Use

## Specifications: 48T/96T

**Please read the instructions carefully before use. If you have any questions, please contact us through the following methods: Official hotline: 025-5229-8998 Sales department phone: 13914481711 Technical phone: 15950492658 Contact email: 3224949330@qq.com Company website: www.byabscience.cn For specific shelf life, please see the reagents Box packaging label. Please use the kit within the shelf life.**

When contacting us, please provide the product number and production date (see box label) so that we can serve you more efficiently.

**Nanjing BYabscience technology Co.,Ltd**

**Website: www.byabscience.cn** **Official hotline: 025-5229-8998** **Supervision phone number:**

**Kit performance Detection range: 1 pmol/mL-32 pmol/mL.**

**Sensitivity: The lowest detectable dose is less than 0.1 pmol/mL.**

**Precision: intra-batch variation coefficient CV% is less than 10%; inter-batch variation coefficient CV% is less than 15%. Recovery rate: The recovery rate is between 85%-115%.**

**Specificity: This kit recognizes native and plant gibberellin 6 (GA6), with no crossover with structural analogs. Stability: Stored at 2℃-8℃, validity period is 6 months.**

**Purpose: Used to detect the concentration of phytogibberellin 6 (GA6) in plant samples.**

**Shelf life: Stored at 2℃-8℃, valid for 6 months.**

## Experimental principle

This kit uses a competitive enzyme-linked immunosorbent assay (ELISA). In the microwell enzyme plate pre-coated with anti-phytogibberellin 6 (GA6) antibody (solid-phase antibody), add the phytogibberellin 6 (GA6) calibrator and the sample to be tested, and then add HRP-labeled phytogibberellin 6 (GA6) calibrator and the sample to be tested. Mycin 6 (GA6) antigen (enzyme-labeled antigen), after incubation and sufficient washing, removes unbound components, and forms an immune complex of solid-phase antibody-enzyme-labeled antigen on the solid surface of the microplate. Add substrates A and B. Under the catalysis of HRP, the substrate produces a blue product. Under the action of the stop solution, it is finally converted into yellow. The absorbance (OD value) is measured at a wavelength of 450nm with a microplate reader. The absorbance (OD value) is related to

The concentration of phytogibberellin 6 (GA6) in the sample to be tested is negatively correlated. By fitting the calibrator curve, the concentration of plant gibberellin 6 (GA6) in the sample can be calculated.

**Kit components and storage: Store unopened kits at 2-8 degrees Celsius. Do not use expired kits.**

| Components | **48-well configuration** | **96-well configuration** | Store after opening |
|---|---|---|---|
| Pre-coated enzyme plate | 48T | 96T | **2-8℃14 days** |
| Standard product | 0.3mL*6 tubes | 0.3mL*6 tubes | **2-8℃14 days** |
| sample diluent | 3ml | 6ml | 2-8℃180 days |
| HRP labeled antigen | 5ml | 10ml | **2-8℃14 days** |
| Chromogenic substrate A | 3ml | 6ml | 2-8℃180 days |
| Chromogenic substrate B | 3ml | 6ml | 2-8℃180 days |
| stop solution | 3ml | 6ml | 2-8℃180 days |
| **20×Lotion** | 15ml | 25ml | 2-8℃180 days |
| sealing film | 2 sheets | 2 sheets | |
| manual | 1 serving | 1 serving | |
| Ziplock bag | 1 | 1 | |

The concentrations of calibrators are: 32, 16, 8, 4, 2, and 0 pmol/mL.

Note: 1: Before use, please check whether the label and quantity of the reagents in the kit are consistent with the table.

2: If the components of the kit need to be used again, please ensure that they have not been contaminated since the last use. 3: If the enzyme plate is not used up in a single time, remember to seal it and store it at 2-8°C.

**Prepare your own test equipment required for the test (not provided, but can assist in**

1) Microplate reader capable of detecting absorbance at 450 nm 2) Pipette, pipette tip, and sample addition tank 3) 37°C incubator or water bath 4) Test tubes, centrifuge tubes,

measuring cylinders, etc. for preparing

reagents 5) Distilled water or deionized

water Ionized water

6) Vortex shaker, microplate shaker

**Notes 1) For scientific research use only, not for clinical diagnosis.**

2) Use within the validity period marked on the kit. Expired products must not be used.

3) Do not mix with kits or components from other manufacturers. Use the sample diluent provided with the kit.

4) If the sample value is higher than the highest standard concentration value, please dilute the sample appropriately and then re-measure.

5) Human anti-mouse and other heterophilic antibodies present in the sample to be tested will interfere with the test results. Please eliminate this factor before testing.

6) The test results obtained by other methods are not directly comparable to the test results of this kit.

7) Please wear a lab coat and latex gloves for protection during the test. Especially when testing blood or other body fluid samples, please follow the national biological laboratory safety protection regulations.

8) Carry out incubation strictly according to the specified time and temperature to ensure accurate results. All reagents must reach room temperature 20-25°C before use. Store reagents refrigerated immediately after use.

9) Improper plate washing can lead to inaccurate results. Make sure to absorb as much liquid as possible from the wells before adding substrate. Do not allow the microwells to dry out during incubation.

10) Eliminate residual liquid and fingerprints on the bottom of the plate, otherwise it will affect the OD value.

11) The substrate chromogenic solution should be colorless or very light in color.

12) Avoid cross-contamination of reagents and specimens to avoid erroneous results.

13) Avoid direct exposure to strong light during storage and incubation.

14) The microplate reader used for detection needs to be equipped with a filter capable of detecting a wavelength of 450±10nm, and the optical density range is between 0-3.5. It is recommended to preheat 15 minutes in advance before use.

15) The EP tubes and tips used in the test are single-use and are strictly prohibited from mixing.

## Sample preparation and storage

The following lists only general guidelines for sample collection and preservation. During the collection and storage of all samples, sodium azide must not be used as a preservative. If the sample is not analyzed immediately, it should be aliquoted and stored frozen, and repeated freezing and thawing should be avoided.

**Plant tissue: 1. Rinse the tissue with pre-cooled PBS (0.01M, PH7.4) to remove residual liquid or impurities on the surface (lysed impurities in the homogenate will affect the measurement results).**

2. Weigh the tissue block and cut it into the smallest possible pieces to allow for complete homogenization.

3. Add an appropriate amount of pre-cooled PBS (generally at a weight-to-volume ratio of 1:9, for example, 1g of tissue sample corresponds to 9mL of PBS. The specific volume can be adjusted appropriately according to experimental needs and recorded. It is recommended to add protease inhibitor to the PBS agent), homogenize thoroughly with a glass homogenizer on ice or in an ice bath. In order to further lyse tissue cells, the homogenate can be sonicated or frozen and thawed repeatedly.

4. Pour the homogenate into a centrifuge tube, centrifuge at 5000×g for 5 minutes at 2-8°C, collect the supernatant, and store it at -20°C or -80°C. Avoid repeated freezing and thawing.

## Reagent preparation 1. Before use, all components must be rewarmed for at least 60 minutes to ensure sufficient rewarming to room temperature.

2. Concentrated washing liquid: The concentrated washing liquid taken out from the refrigerator will produce crystals. This is a normal phenomenon. Heating in a water bath will completely dissolve the

crystals. Concentrated detergent and distilled water, dilute 1:20, that is, 1 part of concentrated detergent, add 19 parts of distilled water.

3. Substrate: Substrate solutions A and B, mix thoroughly at a volume of 1:1 before use, and use within 15 minutes after mixing.

**Operating procedures: Return all reagents and components to room temperature first. It is recommended to do duplicate holes for standards, quality control materials and samples.**

1. Prepare the working solution of various components of the kit according to the method described in the previous instructions.

2. Take out the required slats from the aluminum foil bag, seal the remaining slats in a ziplock bag and return it to the refrigerator.

3. Set up standard wells, blank wells and sample wells. Add 50 µL of standards of different concentrations to each standard well. Do not add any standard to the blank well. Add 50 µL of the sample to be tested to the sample well.

4. In addition to the blank wells, add 100 µL of horseradish peroxidase (HRP)-labeled detection antigen to the standard wells and sample wells. 5. Cover the reaction plate with sealing film and incubate it in a 37°C water bath or incubator for 60 minutes.

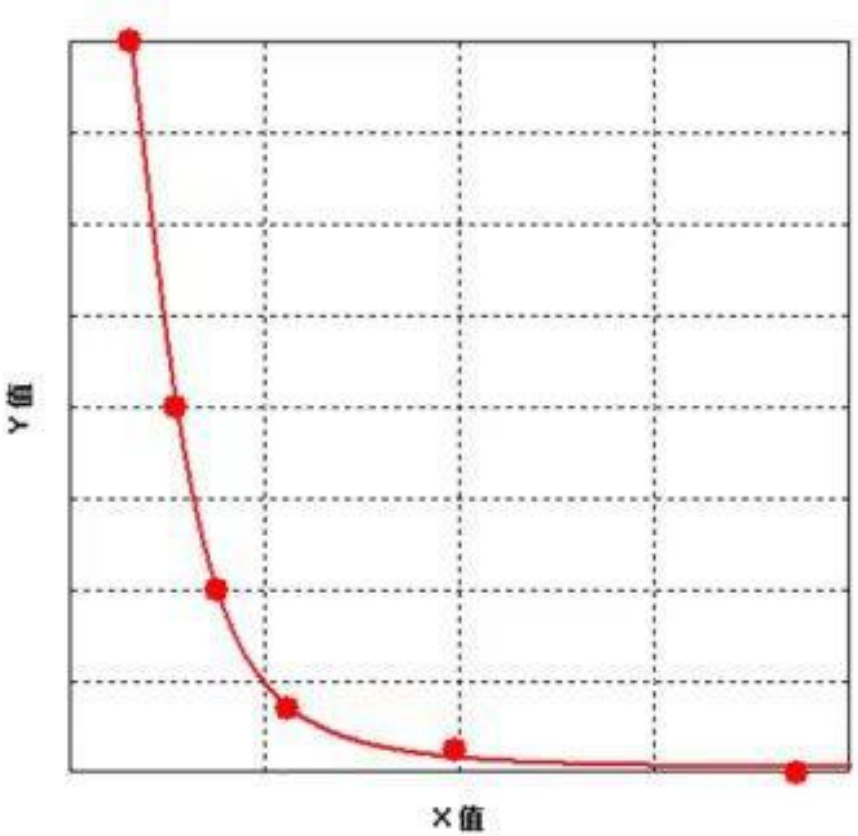
6. Uncover the sealing film, discard the liquid, pat dry on absorbent paper, fill each well with washing liquid, let it stand for 20 seconds, shake off the washing liquid, pat dry on absorbent paper, repeat this 5 times. If you use an automatic plate washer, please wash the plate according to the plate washer operating procedure. Adding a soaking program for 30 seconds can improve the detection accuracy. After washing the plate and before adding substrate, pat the reaction plate dry on clean, lint-free paper. (Tip: In order to obtain ideal experimental results, the residual liquid must be completely removed. After washing the plate, please proceed to the next step immediately and do not let the microplate dry.) 7. Mix substrates A and B at a volume of 1:1 Mix thoroughly and add 100 µL of substrate mixture to all wells. Cover the reaction plate with sealing film and incubate in a 37°C water bath or incubator for 15 minutes.

8. Add 50 μL of stop solution to all wells, and read the absorbance (OD value) of each well on a 450-wavelength microplate reader.

**[Result calculation]**

9. Use the concentration of the standard substance as the abscissa and the corresponding absorbance (OD value) as the ordinate. Use computer software and four-parameter Logistic curve fitting (4-pl) to create a standard curve equation. Through the absorbance (OD value) of the sample value), use the equation to calculate the concentration value of the sample. [Calculation using ELISA Calc software]

10. If the sample is diluted, the concentration value measured by the above method must be multiplied by the dilution factor to obtain the final concentration of the sample. Note: Experimenters need to establish a standard curve based on their own experiments. For each test, a standard curve must be established for each enzyme plate. The following curves are for reference only!




(Schematic diagram of the music, for reference only)

**[Problem Analysis] If the experimental results are not good, please take pictures of the color development results in time, save the experimental data, keep the used strips and unused reagents, and then contact our company's technical support to solve the problem for you. At the same time, you can also refer to the following information: [Questions and Answers]**

| Problem description | Possible reasons | Corresponding countermeasures Corresponding countermeasures |
|---|---|---|
| standard curve gradient difference | Incorrect liquid aspiration or | Check pipettes and tips |
| | Equilibration time is too short | Ensure sufficient balancing time |
| | Incomplete washing | Ensure the washing time and number of washings and the amount of liquid |
| Very weak or colorless | Incubation time too short | Ensure adequate incubation time |
| | The experimental temperature is incorrect | Use recommended experimental temperatures |
| | Insufficient reagent volume or missing addition | Check the liquid aspiration and addition process to ensure that all reagents are added in sufficient |
| | Incorrect dilution | |
| | Enzyme label inactivation or substrate failure | Mix enzyme conjugate and substrate and check by rapid color development |
| Reading value is low | Microplate reader settings are incorrect | Check the wavelength and filter |
| | | Turn on the microplate reader and preheat it in advance |
| Large coefficient of variation | Adding fluid incorrectly | Check the filling situation |
| High background value | The working concentration of the | Use the recommended dilution |
| | Incomplete washing of enzyme plate | Ensure that each step of cleaning is complete; if using an automatic plate washer, please check whether all outlets are blocked; |
| | The lotion is contaminated | Prepare fresh lotion |
| Low sensitivity | Improper storage of ELISA kits | Store relevant reagents according to |
| | Not terminated before reading | Stop solution should be added to |