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User's Manual

Hygromycin phosphotransferase (HPT) ELISA Kit ELISA Kit

REF **DEIA-NS2403-1**

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This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

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PRODUCT INFORMATION

Intended Use

This kit uses a double-antibody sandwich ELISA method to qualitatively and quantitatively detect the content of HPT in crop samples.

Principles of Testing

This kit uses double-antibody sandwich ELISA to detect HPT transgenic components in samples. Pre-coat the microplate microwell strips with monoclonal antibodies specific for HPT. When a sample containing HPT is added, the HPT antibody pre-coated on the microwell strip captures it to form an antibody-antigen complex (Ag-Ab), and then the antibody (Ab), enzyme-labeled antibody (AbHRP) and antigen-antibody are added The complex combines and finally forms an antibody-antigen-antibody-enzyme-labeled antibody complex (Ab-Ag-Ab-HRP), which is then developed by enzyme-catalyzed TMB substrate reagent, and is read by an enzyme reader after termination. The absorbance value of the sample is positively correlated with the HPT content it contains. By comparing it with the standard curve, the HPT content in the sample can be calculated.

Reagents And Materials Provided

HPT MICROWELL PLATE

HPT standard

Antibody working solution

Enzyme working solution

Substrate solution

Stop solution

20× Concentrated wash solution

Sample extraction solution

Materials Required But Not Supplied

Microplate reader (450 nm)

temperate box

Oscillator

Vortex

centrifuge

Polystyrene centrifuge tube: 1.5 mL

Micropipette: single channel 20uL~200uL, 100μL~1000μL, multi-channel 250uL.

Fresh pure or deionized water

Storage

2-8°C (for sealed box), please do not freeze! See kit label for expiry date

Specimen Collection And Preparation

Disposable tips must be used during experiments and the tips must be replaced when absorbing different reagents.

Ensure that all experimental equipment is clean before the experiment to avoid contamination that interferes with the experimental results.

If the sample cannot be tested immediately after collection, it should be stored at -20°C for no more than 1 week.

Sample dilution factor is 10

Sample Preparation:

1. Take 0.1g of sample and add 1 ml of sample extraction solution for extraction;
2. Centrifuge at 4000 rpm for 5 minutes:
3. Take 100 µL supernatant for analysis.

Reagent Preparation

Solution 1:1× Wash Buffer

Dilute the 20×Concentrated wash solution with deionized water at a volume ratio of 1:19 (1 part of 20×Concentrated wash solution + 19 parts of deionized water)

Used for washing microplates, 1× Wash Buffer can be stored at 4°C for one month.

Solution 2: Standard preparation

Use 1mL Sample extraction solution to dissolve the HPT standard. The concentration of this solution is 32 ppb;

Take 300 µl of HPT standard 32 ppb and add 300 µl of Sample extraction solution. The concentration of this solution is 16 ppb;

Take 300 µl of HPT standard 16 ppb and add 300 µl of Sample extraction solution. The concentration of this solution is 8 ppb:

Take 300 µl of HPT standard 8 ppb and add 300 µl of Sample extraction solution. The concentration of this solution is 4 ppb;

Take 300µl of HPT standard 4ppb and 300 µl of Sample extraction solution. The concentration of this solution is 2 ppb;

After diluting the standard solution, please protect it from light and store it at -20°C for 14 days.

Assay Procedure

Note:

1. Before use, bring the temperature of all reagents and required strips back to room temperature (20-25°C)
2. Return all reagents to 2-8°C immediately after use.
3. Reproducibility in ELISA analysis largely depends on the consistency of plate washing. Correct plate washing operation is an important point in the ELISA assay procedure.
4. During all constant temperature incubation processes, avoid light exposure and seal the microplate with a cover film.

Procedure:

1. Take out the required reagents from the refrigerated environment and place them at room temperature (20-25°C) to equilibrate for more than 30 minutes. Note that each liquid reagent must be shaken well before use.
2. Take out the required number of microplates, put the unused microplates into ziplock bags, and store them at 2-8°C.
3. The washing working fluid also needs to be warmed up before use.
4. Numbering: Number the microwells corresponding to the samples and standards in sequence. Make 2 parallel holes for each sample and standard, and record the locations of the standard holes and sample holes.
5. Add standard/sample: Add 100 µL of Sample extraction solution (blank control)/standard/sample to the corresponding microwell, shake gently to mix, and react for 45 minutes at 25°C in a dark environment.
6. Wash the plate: spin dry the liquid in the wells, wash thoroughly 4-5 times with 1× Wash Buffer (Solution 1) 250µL per well, 10 s apart each time, and pat dry with absorbent paper (anything that is not removed after patting dry is Bubbles can be popped with a clean pipette tip).
7. Add Antibody working solution: Add 100 µL of Antibody working solution per well, shake gently to mix, react in a dark environment at 25°C for 30 minutes, remove and repeat plate washing step 6.
8. Enzyme working solution: Add 100uL of Enzyme working solution per well, shake gently to mix, react in a dark environment at 25°C for 30 minutes, remove and repeat plate washing step 6.
9. Color development: Add 100µL per well of Substrate solution and react in a dark environment at 25°C for 10 minutes.
10. Determination: Add 100µL of Stop solution per well, shake gently to mix, set the microplate reader at 450nm (it is recommended to use dual-wavelength 450/630nm detection, and read the data within 5 minutes), and measure the OD value of each well. (If there is no microplate reader, the judgment can be made by visual inspection without adding stop solution)

Calculation

There are two ways to judge the results. The first method can be used for rough judgment, and the second method can be used for quantitative judgment. The calculated value multiplied by the sample dilution factor is the content in the sample. Note: The absorbance value of a sample is directly related to the amount of HPT it contains.

1. Compare the average absorbance value of the sample with the standard value to get the concentration range (ppb). For example, the absorbance value of sample 1 is 0.658, and the absorbance value of sample 2 is 1.658; the absorbance values of the standard products are: 4 ppb is 0.498, 8 ppb is

0.806, 16 ppb is 1.304, and 32 ppb is 2.101. Then the absorbance value of sample 1 The concentration range is 4 ppb-8 ppb, and the concentration range of sample 2 is 16 ppb-32 ppb.

2. Quantitative analysis

a. Calculation of absorbance value of standard product:

The average of the absorbance values of the standard or sample (double wells) - the absorbance value of the sample dilution (background).

b. Drawing and calculation of the standard curve: Draw the standard curve using the absorbance value of the standard as the ordinate and the HPT standard concentration (ppb) as the abscissa. Substitute the absorbance value of the sample into the standard curve and read the concentration corresponding to the sample from the standard curve.

If the professional analysis software of the kit is used for calculation, it will be more convenient for accurate and rapid analysis of a large number of samples.

Precautions

1. If the room temperature is lower than 20°C or the reagents and samples have not returned to room temperature (20-25°C), the OD values of all standards will be low.
2. If the plate wells become dry during the plate washing process, the standard curve will not be linear and the repeatability will be poor. Therefore, you should proceed to the next step immediately after washing the plate and patting it dry.
3. Shake well before adding each reagent.
4. The stop solution is 2M sulfuric acid, avoid contact with
5. Do not use a kit that has expired, nor use any reagents in a kit that has expired. Do not exchange reagents from kits with different lot numbers.
6. Storage conditions: Store the kit at 2-8°C. Do not freeze. Put unused microplates into ziplock bags and reseal them. Standard and Substrate solution are sensitive to light and heat, so direct exposure to light should be avoided.
7. Signs of reagent deterioration: Any color in the chromogenic reagent indicates deterioration of the chromogen and should be discarded.
8. After adding the Substrate solution, it usually takes 15 minutes to develop the color. If the color is lighter, the reaction time can be extended to 20 minutes (or longer), but should not exceed 30 minutes. On the contrary, the reaction time is shortened.
9. The optimal reaction temperature of this kit is 25°C. Temperature that is too high or too low will cause changes in the detection absorbance value and sensitivity.