Version 01-06/24

User's Manual

Ferulic acid ELISA Kit

REF **DEIANJ07**

Σ **96T**

RUO

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 is a competitive enzyme-labeled immunoassay and can be used in quantitative analysis of ferulic acid in wheat.

Principles of Testing

This kit was based on Competitive-ELISA detection method. The microtiter plate provided in this kit has been pre-coated with target. During the reaction, target in the sample or standard competes with a fixed amount of target on the solid phase supporter for sites on the Antibody specific to target. Excess antibody and unbound sample or standard are washed from the plate, and Enzyme Conjugate is added to each microplate well and incubated. Then substrate solution is added to each well. The enzyme-substrate reaction is terminated by the addition of a sulphuric acid solution and the color change is measured spectrophotometrically at a wavelength of 450nm. The concentration of target in the samples is then determined by comparing the OD of the samples to the standard curve.

Reagents And Materials Provided

1. Microtiter plate with 96 wells coated with coupling antigen
2. Spiking standard 25ug/mL, 200ul
3. Standard Dilution Buffer, 35mL
4. 20× Sample Dilution Buffer, 35mL
5. Antibody Solution, 7mL
6. Enzyme Conjugate, 12mL
7. Substrate Solution, 2×6mL
8. Stop Solution, 7mL
9. 20× Wash Solution, 50mL
10. Empty brown tubes, 7
11. Instruction

Materials Required But Not Supplied

1. Microplate reader (wavelength:450nm/620nm)
2. Clean tubes and Eppendorf tubes
3. Precision single and multi-channel pipette and disposable tips
4. Reciprocating oscillator
5. Paper towels or equivalent absorbent material
6. Timer
7. Sodium sulfite (Na_2SO_3)

8. Methanol
9. Deionized or distilled water

Storage

1. All reagents should be stored at 2°C to 8°C for stability.
2. Before using, bring all components to room temperature (20-25°C). Upon assay completion ensure all components of the kit are returned to appropriate storage conditions.
3. The Substrate and Spiking standard is light-sensitive and should be protected from direct sunlight or UV sources.

Specimen Collection And Preparation

1. Homogenize the sample.
2. Weigh 0.5g±0.01g homogeneous wheat samples into a 50mL centrifuge tube. Add 10mL of Sample extraction solution (solution 1), mix well and place it in a reciprocating oscillator. The oscillation frequency is 220 ± 10 r/min, and react at 37 °C for 60 minutes, then centrifuge for 5min at 5000r/min.
3. Draw 50uL of supernatant into 1.5mL centrifuge tube, add 950uL 1× Sample Dilution Buffer (solution 2), mix well.
4. Take 50ul of liquid for detection.

Dilution factor: 400

The user should estimate the concentration of target protein in the test sample, and select a proper dilution factor to make the diluted target protein concentration fall in the optimal detection range of the kit. Dilute the sample with the provided dilution buffer, and several trials may be necessary. The test sample must be well mixed with the Sample Dilution Buffer. And also standard curves and sample should be making in pre-experiment. If samples with very high concentrations, dilute samples with 1× Sample Dilution Buffer.

Note: The extraction efficiency of ferulic acid in samples is affected by temperature and methanol volume fraction. The extraction method can be adjusted by the customer according to the actual situation. But eventually the samples were diluted with 1× sample dilution buffer.

Ferulic acid is sensitive to light, and it should be protected from light during the experiment. Try to use brown centrifuge tubes.

Reagent Preparation

Solution 1: Sample extraction solution

Dilute the Methanol with deionized water in the volume ratio of 6:4 and mix them thoroughly. (e.g. Add 60mL Methanol into 40mL deionized water). Adding 0.2g/L sodium sulfite (Na_2SO_3) into the Sample extraction solution is recommended.

Solution 2: 1× Sample Dilution Buffer

Dilute the 20× Sample Dilution Buffer with deionized water in the volume ratio of 1:19. (e.g. Add 10mL 20× Sample Dilution Buffer into 190mL deionized water.)

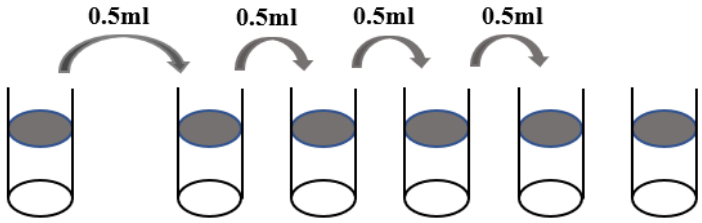
Solution 3: 1× Wash solution

Dilute the 20× Wash solution with deionized water in the volume ratio of 1:19. (e.g. Add 10mL 20× Wash solution into 190mL deionized water.)

Solution 4: Standard solution

Add 9.72 ul Spiking standard (25 µg/ml) into a tube with 990.28 ul Standard Dilution Buffer to prepare a 243 ng/ml standard solution.

Pipette 1ml Standard Dilution Buffer into each tube. Use the 243 ng/ml standard solution to produce a dilution series (shown below). Mix each tube thoroughly before the next transfer. Standard Dilution Buffer serves as the zero standard (0 ng/ml).



	Std1	Std2	Std3	Std4	Std5	Std6
Diluent Volume	9.72ul spiking standard+990.28ul	1ml	1ml	1ml	1ml	1ml
Conc. (ng/ml)	243	81	27	9	3	0

Assay Procedure

Take all reagents out at room temperature (20-25°C) for more than 30min. When diluting samples and reagents, they must be mixed completely and evenly. It is recommended to plot a standard curve for each test.

1. Set standards, test samples on the pre-coated plate respectively, and then, records their positions. It is recommended to measure each standard and sample in duplicate.
2. **Add Sample and Antibody:** Add 50ul of Standard or Sample per well. The blank well is added with Standard Dilution Buffer. Immediately add 50ul Antibody Solution into each well. Cover with the Plate sealer and incubate for 30 minutes at 2-8°C. (Solutions are added to the bottom of microplate well, avoiding inside wall touching and foaming as much as you can.)
3. **Wash:** Remove the cover, and wash plate 5 times with wash solution, and let the wash solution stay in the wells for 10 seconds each time. After the last wash, remove any remaining wash solution by aspirating or decanting.
4. **Add Enzyme Conjugate:** Add 100ul Enzyme Conjugate into each well. Cover it with a new Plate sealer. Incubate for 30 minutes at 25°C.
5. **Wash:** Remove the cover and wash plate 5 times with wash solution, and let the wash solution stay in the wells for 10 seconds each time.
6. **Coloration:** Add 100ul Substrate Solution into each well, cover the plate and incubate at 25°C in dark for 15 minutes. Note: The reaction time can be shortened or extended according to the actual color change, but not more than 30 minutes. You can terminate the reaction when apparent gradient appeared in standard wells.)
7. **Stop:** Add 50ul Stop Solution into each well. The color will turn yellow immediately. The adding order of

Stop Solution should be as the same as the Substrate Solution.

8. **OD Measurement:** Read the O.D. absorbance at 450nm (recommended to use dual-wavelength 450/620nm detection) in Microplate Reader immediately after adding the stop solution.

Calculation

Percentage absorbance: The mean values of the absorbance values obtained from the standards and the samples are divided by the absorbance value of the standard (0 ng/ml) and multiplied by 100%.

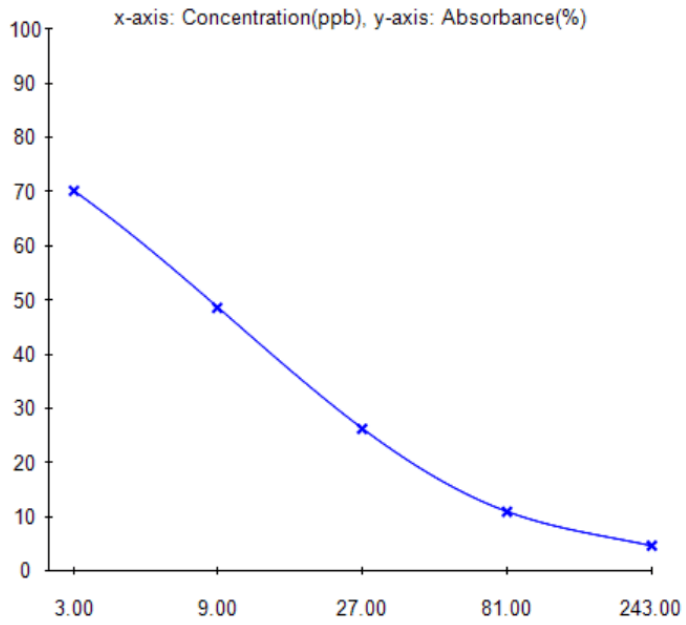
Absorbance (%) = (B/B_0) *100%

B - absorbance of standards or samples

B_0 - absorbance of 0 ng/ml

Note: The standard curve is drawn by taking the percent absorbance of the standard as the ordinate and the logarithm of the ferulic acid standard concentration (ng/ml or ppb) as the abscissa. The percent absorbance of the sample is substituted into the standard curve, and the concentration corresponding to the sample is read from the standard curve, and the corresponding dilution factor is multiplied by the actual concentration of ferulic acid in the sample. If professional analysis software is used for calculation, it is more convenient for accurate and rapid analysis of a large number of samples.

Typical Standard Curve



Spline

Standards
Conc.(ppb) / A(Mean)

3.00 1.614
(Coeff. of Var.: 0.2%)

9.00 1.117
(Coeff. of Var.: 5.1%)

27.00 0.603
(Coeff. of Var.: 1.3%)

81.00 0.251
(Coeff. of Var.: 1.7%)

243.00 0.107
(Coeff. of Var.: 0.7%)

50% inhibition: 8.4

Precision

Intra-Assay: CV<10%

Inter-Assay: CV<10%

Detection Range

0-243 ng/ml

Sensitivity

3 ng/ml

Precautions

1. Room temperature below 20 °C or reagents and samples not returned to room temperature (20-25 °C) will result in low OD values.
2. If the plate well is dry during the washing process, the standard curve is not linear and the repeatability is not good. Therefore, the next step should be taken immediately after the plate is patted dry.
3. Shake the reagents before use and avoid bubbles when mixing.
4. Keep your skin away from the stop solution for it is 2M H_2SO_4 solution.
5. Please do not mix the reagents in different kits of our company. Do not mix reagents from other manufacturers.
6. Storage TMB reagents avoid light.
7. To inspect the validity of experiment operation and the appropriateness of sample dilution proportion, pilot experiment using standards and a small number of samples is recommended.