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Plant zeatin riboside (ZR) ELISA Kit

96 Tests

Catalogue Number:SL0023PI

Store all reagents at 2-8°C

Validity Period: six months

For samples:

In serum, plasma, culture media or any biological fluid.

FOR RESEARCH USE ONLY !

NOT FOR THERAPEUTIC OR DIAGNOSTIC APPLICATIONS !

PLEASE READ THROUGH ENTIRE PROCEDURE BEFORE BEGINNING !

Plant zeatin riboside (ZR) ELISA Kit

FOR RESEARCH USE ONLY

Drug Names

Generic Name: **Plant zeatin riboside (ZR) ELISA Kit**

Purpose

Our Plant zeatin riboside (ZR) ELISA kit is to assay ZR levels in Plant serum, plasma, culture media or any biological fluid.

Principle

This ELISA kit uses Sandwich-ELISA as the method. The Microelisa stripplate provided in this kit has been pre-coated with an antibody specific to ZR. Standards or samples are added to the appropriate Microelisa stripplate wells and combined to the specific antibody. Then a Horseradish Peroxidase (HRP)-conjugated antibody specific for ZR is added to each Microelisa stripplate well and incubated. Free components are washed away. The TMB substrate solution is added to each well. Only those wells that contain ZR and HRP conjugated ZR antibody will appear blue in color and then turn yellow after the addition of the stop solution. The optical density (OD) is measured spectrophotometrically at a wavelength of 450 nm. The OD value is proportional to the concentration of ZR. You can calculate the concentration of ZR in the samples by comparing the OD of the samples to the standard curve.

Materials provided with the kit

	Materials provided with the kit	96 determinations	Storage
1	User manual	1	R.T.
2	Closure plate membrane	2	R.T.
3	Sealed bags	1	R.T.
4	Microelisa stripplate	1	2-8°C
5	Standard: 450 ng/L	0.5ml×1 bottle	2-8°C
6	Standard diluent	1.5ml×1 bottle	2-8°C
7	HRP-Conjugate reagent	6ml×1 bottle	2-8°C

8	Sample diluent	6ml×1 bottle	2-8°C
9	Chromogen Solution A	6ml×1 bottle	2-8°C
10	Chromogen Solution B	6ml×1 bottle	2-8°C
11	Stop Solution	6ml×1 bottle	2-8°C
12	wash solution	20ml (30X)×1bottle	2-8°C

Sample collection and storages

1. Can't detect the samples which contain NaN₃, because NaN₃ inhibits HRP activity of the horseradish peroxidase.
2. Extract as soon as possible after Specimen collection, Extracted according to the relevant literature.

Cell culture supernates and plant exact fluids - Remove particulates by centrifugation and assay immediately or aliquot and store samples at -20°C or -80°C. Avoid repeated freeze-thaw.

Notes:

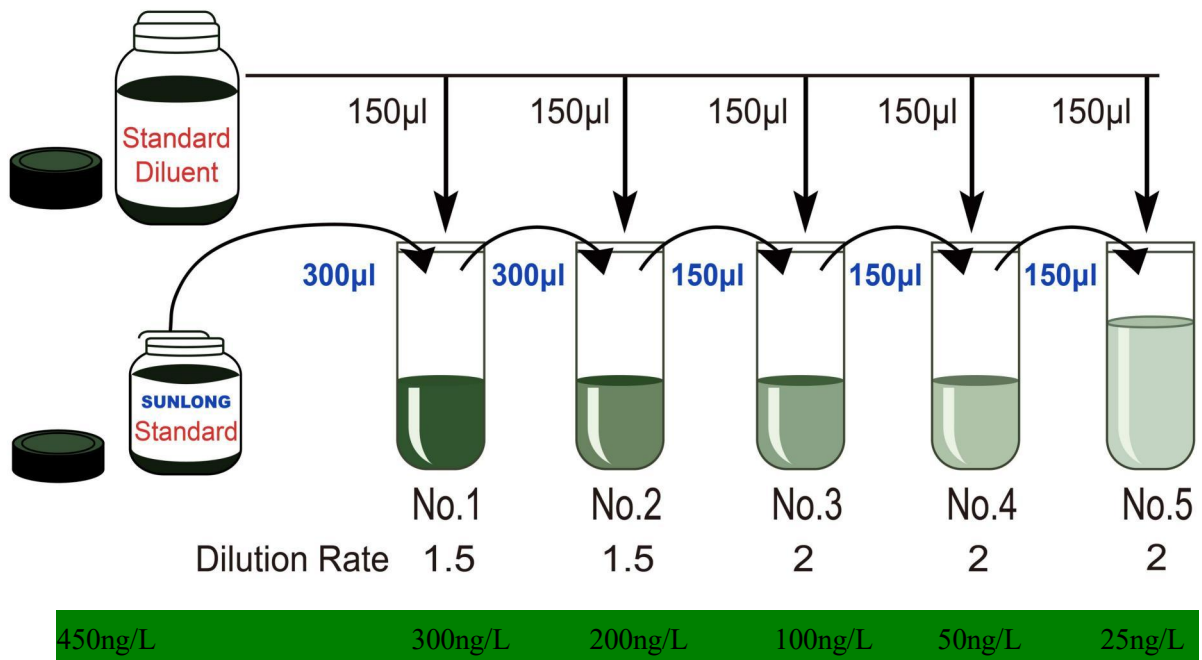
Sample extraction and ELISA assay should be performed as soon as possible after sample collection. The samples should be extracted according to the relevant literature. If ELISA assay can not be performed immediately, samples can be stored at -20 °C .Repeated freeze-thaw cycles should be avoided.

Procedure

1. Dilution of Standards

Dilute the standard by small tubes first, then pipette the volume of 50ul from each tube to microplate well, each tube use two wells, total ten wells.

300ng/L	Standard No.1	300µl Original Standard + 150µl Standard diluents
200ng/L	Standard No.2	300µl Standard No.1 + 150µl Standard diluents
100ng/L	Standard No.3	150µl Standard No.2 + 150µl Standard diluent
50ng/L	Standard No.4	150µl Standard No.3 + 150µl Standard diluent
25ng/L	Standard No.5	150µl Standard No.4 + 150µl Standard diluent



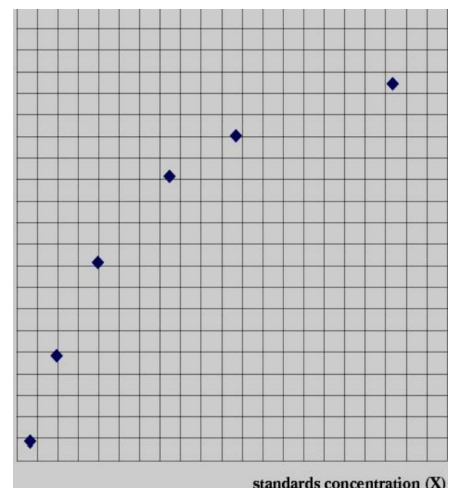
2. In the Microelisa stripplate, leave a well empty as blank control. In sample wells, 40µl Sample dilution buffer and 10µl sample are added (dilution factor is 5). Samples should be loaded onto the bottom without touching the well wall. Mix well with gentle shaking.
3. Incubation: incubate 30 min at 37°C after sealed with Closure plate membrane.
4. Dilution: dilute the concentrated washing buffer with distilled water (30 times for 96T and 20 times for 48T).
5. Washing: carefully peel off Closure plate membrane, aspirate and refill with the wash solution. Discard the wash solution after resting for 30 seconds. Repeat the washing procedure for 5 times.
6. Add 50 µl HRP-Conjugate reagent to each well except the blank control well.
7. Incubation as described in Step 3.
8. Washing as described in Step 5.
9. Coloring: Add 50 µl Chromogen Solution A and 50 µl Chromogen Solution B to each well, mix with gently shaking and incubate at 37 °C for 15 minutes. Please avoid light during coloring.
10. Termination: add 50 µl stop solution to each well to terminate the reaction. The color in the well should change from blue to yellow.
11. Read absorbance O.D. at 450nm using a Microtiter Plate Reader. The OD value of the blank control well is set as zero. Assay should be carried out within 15 minutes after adding stop solution.

Notes:

1. Store the kit at 4 ° C upon receipt. The kit should be equilibrated to room temperature before the assay. Remove any unneeded strips from Plant ZR Antibody-Coated plate, reseal them in zip-lock foil and keep at 4 ° C.
2. Precipitates may appear in concentrated washing buffer. Please heat the buffer to dissolve all the precipitates, which will not affect the results.
3. Accurate pipette should be used to avoid experimental error. Samples should be added to the Microplate in less than 5 minutes. If a large number of samples are included, multiple channel pipette is recommended.
4. Standard curve should be included in every assay. Replicate wells are recommended. If the OD value of the sample is greater than the first well of standards, please dilute the sample (n times) before test. When calculating the original ZR concentration, please multiply the total dilution factor (XnX5).
5. In order to avoid cross-contamination, Microplate sealers are for one-time use only.
6. Please keep Substrate away from light.
7. All the operation should be accordance with the manufacturer's instructions strictly. The results determined by the Microtiter Plate Reader.
8. All the samples, washing buffer and wastes should be treated as infectious agents.
9. Reagents from different lots should not be mixed.

Calculation of Results

Known concentrations of Plant ZR Standard and its corresponding reading OD is plotted on the log scale (x-axis) and the log scale (y-axis) respectively. The concentration of Plant ZR in sample is determined by plotting the sample's O.D. on the Y-axis. The original concentration is calculated by multiplying the dilution factor.



This diagram is for reference only

Precision

Intra-assay Precision (Precision within an assay): 3 samples with low, middle and high level Plant ZR were tested 20 times on one plate, respectively.

Inter-assay Precision (Precision between assays): 3 samples with low, middle and high level Plant ZR were tested on 3 different plates, 8 replicates in each plate.

$$CV(\%) = SD/\text{mean} \times 100$$

Intra-Assay: $CV < 10\%$

Inter-Assay: $CV < 12\%$

Assay range

10 ng/L - 300 ng/L

Sensitivity:

2.6 ng/L

Storage and validity

- 1 . Storage: 2-8°C.
- 2 . Duration: 6 months