PRODUCT INFORMATION & MANUAL

Plant Root Activity Assay Kit (Colorimetric)

NBP3-25921

For research use only.
Not for diagnostic or therapeutic procedures.

www.novusbio.com - P: 303.730.1950 - P: 888.506.6887 - F: 303.730.1966 - technical@novusbio.com

Plant Root Activity Assay Kit (Colorimetric)

Catalog No: NBP3-25921

Method: Colorimetric method

Specification: 100 Assays (Can detect 43 samples without duplication)

Instrument: Spectrophotometer

Average intra-assay CV (%): 1.0

Average inter-assay CV (%): 3.0

Average recovery rate (%): 103

- ▲ This kit is for research use only.
- ▲ Instructions should be followed strictly, changes of operation may result in unreliable results.
- ▲ Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

General information

▲Intended use

This kit can be used to measure root activity of all plant samples.

▲Detection principle

Plant roots are active organs of absorption and biosynthesis. Root growth and metabolism, namely root activity, will directly affect the growth, nutritional status and final yield of above-ground parts of plants, and is one of the important physiological indicators of plant growth.

The detection principle of the kit is to use 2,3,5-triphenyltetrazolium chloride (TTC) as the substrate, incubate for 1 to 3 hours, the dehydrogenase in the root can reduce TTC and generate 1,3,5-tiphenylformazan (TTF) which is insoluble in water, then TTF will be extracted from the root with organic solvent (ethyl acetate or acetone, etc.) By detecting the absorbance at 485nm, the amount of TTC reduction can be calculated, which represents the dehydrogenase activity and is used as an index of plant root activity. The kit is used for quantitative determination of plant root activity or dehydrogenase activity.

▲Kit components & storage

Item	Component	Specification	Storage
Reagent 1	Buffer Solution	25 mL × 1 vial	-20℃ , 12 months
Reagent 2	Chromogenic Agent	Powder × 1 vial	-20℃ , 12 months, shading light
Reagent 3	Reducing Reagent	Powder × 1 vial	-20℃ , 12 months, shading light
Reagent 4	Stop Solution	25 mL × 1 vial	-20℃ , 12 months
Note: The reagents must be stored strictly according to the preservation conditions in the above table. The reagents in different kits cannot be mixed with each other.			

▲Materials prepared by users

Instruments

Spectrophotometer (485 nm), 37℃ incubator, High-speed freezing centrifuge

Reagents:

Double distilled water, Ethyl Acetate

⚠ Safety data

Some of the reagents in the kit contain dangerous substances. It should be avoided to touch the skin and clothing. Wash immediately with plenty of water if touching it carelessly. All the samples and waste material should be treated according to the relevant rules of laboratory's biosafety.

⚠ Precautions

Before the experiment, please read the instructions carefully, and wear gloves and work clothes.

▲ The key points of the assay

1. The reagent 2 should be stored with shading light and the prepared reagent 2 working solution should be used up within 1 week.
2. The experiment should be carried out in the fume cupboard.
3. It is recommended to use fresh samples.

Pre-assay preparation

▲ Reagent preparation

1. Heat the reagent 1 in 60C until clear if it is crystallised.
2. Preparation of reagent 1 working solution:
   Dilute reagent 1 with double distilled water at a ratio of 1:9. The prepared solution should be stored at 2-8℃ with shading light for 7 days.
3. Preparation of reagent 2 working solution:
   Dissolve 0.4 g reagent 2 in 100 mL of double distilled water. The prepared solution should be stored at 2-8℃ with shading light for 7 days. Discard if it turns red.
4. Preparation of reagent 2 reaction working solution:
   Dilute reagent 1 working solution with reagent 2 working solution at a ratio of 1:1. Prepare the fresh needed amount before use and the prepared solution should be used up within the same day.
5. Preparation of reagent 3 working solution:
   Dissolve 20mg reagent 3 with 0.5 mL double distilled water. The prepared solution can be stored at 2-8℃ for 1 day.
6. Preparation of reagent 4 working solution:
   Dilute reagent 4 with double distilled water at a ratio of 1:4(add reagent 4 to double distilled water).Prepare the fresh needed amount before use and the prepared solution can be stored at 2-8℃ for 7 days.
7. Preparation of 100 μg/mL TTC standard solution:
   Take 0.2 mL of reagent 2 working solution to a 10 mL EP tube and mix fully with 50 μL reagent 3 working solution. It produces water-insoluble red particles, then add 8 mL of ethyl acetate, completely mix and dissolve the particles, and stand for stratification (To ensure that the particles completely dissolved). The organic phase (red solution in top layer) is the 100ug/mL TTC standard solution.

▲ Sample preparation

Select the root of the plant (preferably the root hair region), rinse it thoroughly with double distilled water, and use absorbent paper to absorb moisture. Cut with scissors to a length of 0.5-2 cm, weigh 0.1-0.3g of each sample and place it in a 10 mL EP tube for use.

▲Dilution of sample

It is recommended to take 2~3 samples with expected large difference to do pre-experiment before formal experiment and dilute the sample according to the result of the pre-experiment and the detection range (0-0.67 U/g wet weigh).

The recommended dilution factor for different samples is as follows (for reference only)

Sample type	Dilution factor
cymbidium tissue homogenate	1
black nightshade tissue homogenate	1
Stone step grass tissue homogenate	1
bitter fleabane tissue homogenate	1
hemlock chervil tissue homogenate	1
oilseed rape tissue homogenate	1

Note: The diluent is ethyl acetate.

Assay protocol

▲Plate set up

	1	2	3	4	5	6	7	8	9	10	11	12
A	S1	S1'	S9	S9'	S17	S17'	S25	S25'	S33	S33'	S41	S41'
B	S2	S2'	S10	S10'	S18	S18'	S26	S26'	S34	S34'	S42	S42'
C	S3	S3'	S11	S11'	S19	S19'	S27	S27'	S35	S35'	S43	S43'
D	S4	S4'	S12	S12'	S20	S20'	S28	S28'	S36	S36'	S44	S44'
E	S5	S5'	S13	S13'	S21	S21'	S29	S29'	S37	S37'	S45	S45'
F	S6	S6'	S14	S14'	S22	S22'	S30	S30'	S38	S38'	S46	S46'
G	S7	S7'	S15	S15'	S23	S23'	S31	S31'	S39	S39'	S47	S47'
H	S8	S8'	S16	S16'	S24	S24'	S32	S32'	S40	S40'	S48	S48'

[Note]: S1-S48: Sample wells; S1 '-S48' : control wells

▲Detailed operation steps

1. The preparation of standard curve

Dilute 100 μg/mL standard solution with ethyl acetate to a serial concentration. The recommended dilution gradient is as follows: 0, 25, 50, 75, 100, 150, 200 μg. Reference is as follows:

Number	Standard concentrations (μg)	100 μg/mL TTC Standard solution (mL)	ethyl acetate (mL)
A	0	0	5.00
B	25	0.25	4.75
C	50	0.5	4.50
D	75	0.75	4.25
E	100	1.00	4.00
F	150	1.50	3.50
G	200	2.00	3.00

2. The measurement of samples

(1) Standard tube: Take 100 μg/ml TTF upper red solution 0, 0.25, 0.5, 0.75, 1.0, 1.5, 2.0 ml into a 15 mL centrifuge tube, and then add ethyl acetate 5.0, 4.75, 4.5, 4.25, 4.0, 3.5, 3.0 ml, respectively.Take 200 uL and measure the OD value of each tube at 485 nm.

Sample/ Control tube: weigh 0.1-0.3g of sample and add 5 mL EP tube.

(2) Add 1 mL of reagent 4 working solution and 4 mL of reagent 2 reaction working solution in sequence to control tube. Add 4 mL of reagent 2 reaction working solution to sample tube.

(3) Mix fully and incubate at 37°C for 3 h with shading light.

(4) Add 1 mL of reagent 4 working solution into sample tube.

(5) Take out the sample, dry the surface water and cut it into pieces. Homogenate with 1 mL of ethyl acetate until the red substance in the root sample is completely extracted. Then, dilute the homogenate to 5 mL with ethyl acetate.

(6) Mix fully, take 200μL of sample and measure the absorbance value of the sample at 485 nm with spectrophotometer (with a diameter of 1.0 cm in the cuvette and a series of standard tubes zeroed with a blank tube).

▲Summary operation table

Standard tube

TTC weight (μg)	0	25	50	75	100	150	200
100 μg/mL TTC standard solution (mL)	0	0.25	0.50	0.75	1.00	1.50	2.00
Ethyl acetate (mL)	5.00	4.75	4.50	4.25	4.00	3.50	3.00
Take 200 uL and measure the OD value of each tube at 485 nm.							

Sample/ Control tube

	Control tube	Sample tubel
Sample (g)	0.1-0.3	0.1-0.3
Reagent 4 working solution (mL)	1	
Reagent 2 reaction working solution (mL)	4	4
Mix fully and incubate at 37°C for 3 h with shading light.		
Reagent 4working solution (mL)		1
Take out the sample, dry the surface water and cut it into pieces. Homogenate with 1 mL of ethyl acetate until the red substance in the root sample is completely extracted. Then, dilute the homogenate to 5 mL with ethyl acetate.		
Mix fully, take 200μL of sample and measure the absorbance value of the sample at 485 nm with spectrophotometer (with a diameter of 1.0 cm in the cuvette and a series of standard tubes zeroed with a blank tube).		

Calculation

Plot the standard curve by using OD value of standard and correspondent concentration as y-axis and x-axis respectively. Create the standard curve with graph software (or EXCEL). The concentration of the sample can be calculated according to the formula based on the OD value of sample. The standard curve is: y= ax + b.

Definition: 1 g of plant root that catalyzed 1 mg of TTC to generate TTF at 37C in 1 hour is defined as 1 unit.

Plant Root Activity (U/g wet weight)) = $(\Delta A_{485} - b) \div a \times f \div (1000 \times m \times t)$

Note:

y: $OD_{Standard} - OD_{Blank}$ (OD Blank is the OD value when the standard concentration is 0).

x: The concentration of standard.

a: The slope of standard curve.

b: The intercept of standard curve.

ΔA_{485}: $(OD_{Sample} - OD_{Control})$.

f: Dilution factor of sample before test.

m: Weight of tissue, g.

T: The time of incubation, h.

1000: 1 mg = 1000 μg.

Appendix I Data

Example analysis

Weigh 0.1-0.3g stone step grass and carry the assay according to the operation table.

The results are as follows:

standard curve: y = 0.0042 x + 0.0163, the average OD value of the control is 0.076, the average OD value of the sample is 0.537, and the calculation result is:

Plant Root Activity (U/g wet weight)

= (0.537 - 0.076 - 0.0163) ÷ 0.0042 ÷ (1000 × 0.2 × 3) = 0.18 U/g wet weight