（FOR RESEARCH USE ONLY. DO NOT USE IT IN CLINICAL DIAGNOSIS !）

Catalog No：E-BC-K855-S

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

Measuring instrument：Spectrophotometer（485 nm）

Detection range：0-0.67 U/g wet weigh

Elabscience®Plant Root Activity Assay Kit

This manual must be read attentively and completely before using this product.
If you have any problem, please contact our Technical Service Center for help：

Toll-free: 1-888-852-8623
Tell: 1-832-243-6086
Fax: 1-832-243-6017
Email: techsupport@elabscience.com
Website: www.elabscience.com

Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

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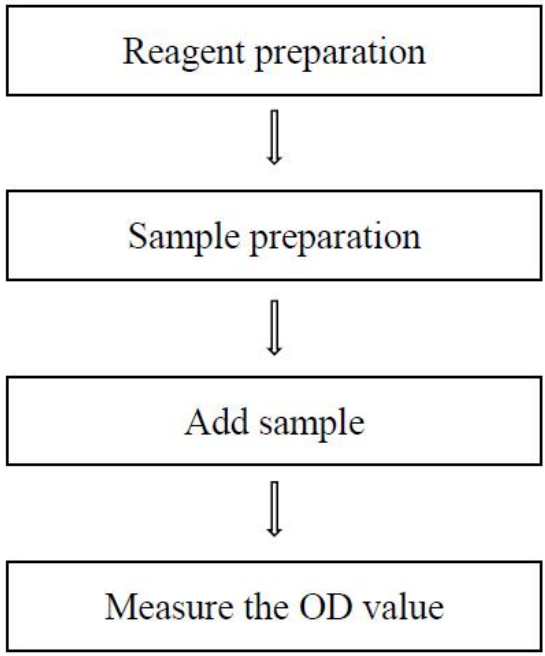
Assay summary



Intended use

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

Detection principle

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	Size (100 Assays)	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. For a small volume of reagents, please centrifuge before use, so as not to obtain sufficient amount of reagents.

Materials prepared by users

Instruments:

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

Reagents:

Double distilled water, Ethyl Acetate

Reagent preparation

① Heat buffer solution in 60℃ until clear if it is crystallised. Equilibrate other reagents to room temperature before use.

② Preparation of buffer working solution：
Dilute 200 μL of buffer solution with 1800 μL of double distilled water, mix well. Store at 2-8°C for 7 days.

③ Preparation of chromogenic working solution：
Dissolve 0.4 g chromogenic agent with 100 mL of double distilled water, mix well. Store at 2-8 °C for 7 days protected from light. Discard if it turns red.

④ Preparation of chromogenic reaction working solution：
Dilute 2 mL of buffer working solution with 2 mL of chromogenic working solution, mix well. The chromogenic reaction working solution should be prepared on spot and used up within the same day.

⑤ Preparation of reducing working solution：
Dissolve 20 mg reducing reagent with 0.5 mL of double distilled water, mix well. Store at 2-8 °C for 7 days. The reducing working solution should be prepared on spot.

⑥ Preparation of stop working solution：
For each well, prepare 1000 μL of stop working solution (mix well 200 μL of stop solution and 800 μL of double distilled water).

⑦ Preparation of 100 μg/mL TTC standard solution：
Take 0.2 mL of chromogenic working solution to a 10 mL EP tube and mix

fully with 50 μL reducing 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 100 ug/mL TTC standard solution

⑧ The preparation of standard curve：

Always prepare a fresh set of standards. Discard working standard dilutions after use.

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. Reference is as follows:

Item	①	②	③	④	⑤	⑥	⑦
Concentration (μg)	**0**	**25**	**50**	**75**	**100**	**150**	**200**
100 μg/mL TTC standard (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

Sample preparation

① Sample preparation

Tissue sample:

① 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.

② Cut with scissors to a length of 0.5-2 cm, weigh 0.1-0.3 g of each sample and place it in a 10 mL EP tube for use.

② Dilution of sample

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

Sample type	Dilution factor
Cymbidium	1
Black nightshade	1
Stone step grass	1
Bitter fleabane	1
Hemlock chervil	1
Oilseed rape	1

Note: The diluent is ethyl acetate. For the dilution of other sample types, please do pretest to confirm the dilution factor

The key points of the assay

① The chromogenic agent should be stored with shading light and the prepared chromogenic working solution should be used up within 1 week.

② The experiment should be carried out in the fume hood.

③ It is recommended choose fresh plant roots.

Operating steps

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 μL and measure the OD value of each tube at 485 nm

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

② Add 1 mL of stop working solution and 4 mL of chromogenic reaction working solution in sequence to control tube. Add 4 mL of chromogenic reaction working solution to sample tube.

③ Mix fully and incubate at 37℃ for 3 h with shading light.

④ Add 1 mL of stop 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 200 μL 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

The standard curve:

1. Average the duplicate reading for each standard.

2. Subtract the mean OD value of the blank (Standard #①) from all standard readings. This is the absoluted OD value.

3. Plot the standard curve by using absoluted OD value of standard and correspondent concentration as y-axis and x-axis respectively. Create the standard curve (**y = ax + b**) with graph software (or EXCEL).

The sample:

Tissue sample:

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

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

[Note]

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 Performance Characteristics

1. Parameter:

Intra-assay Precision

Three cymbidium samples were assayed in replicates of 20 to determine precision within an assay (CV = Coefficient of Variation).

Parameters	Sample 1	Sample 2	Sample 3
Mean (U/g wet weight)	0.08	0.24	0.52
%CV	1.2	0.9	0.9

Inter-assay Precision

Three cymbidium samples were assayed 20 times in duplicate by three operators to determine precision between assays.

Parameters	Sample 1	Sample 2	Sample 3
Mean (U/g wet weight)	0.08	0.24	0.52
%CV	2.7	3.2	3.1

Recovery

Take three samples of high concentration, middle concentration and low concentration to test the samples of each concentration for 6 times parallelly to get the average recovery rate of 103%.

	Standard 1	Standard 2	Standard 3
Expected Conc. （μg）	35	95	163
Observed Conc. （μg）	35.4	98.8	169.5
recovery rate(%)	101	104	104

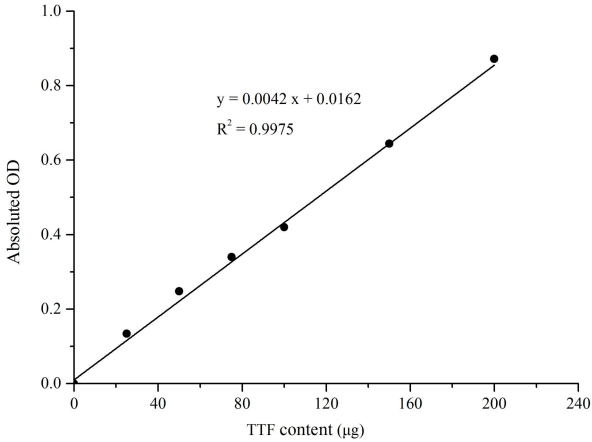
Sensitivity

The analytical sensitivity of the assay is 0 U/g wet weight. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 20 times, and calculating the corresponding concentration.

2. Standard curve:

As the OD value of the standard curve may vary according to the conditions of the actual assay performance (e.g. operator, pipetting technique or temperature effects), so the standard curve and data are provided as below for reference only:

Standard quality (μg)	0	25	50	75	100	150	200
OD Value	0	0.134	0.248	0.340	0.380	0.644	0.872



Appendix II Example Analysis

Example analysis：

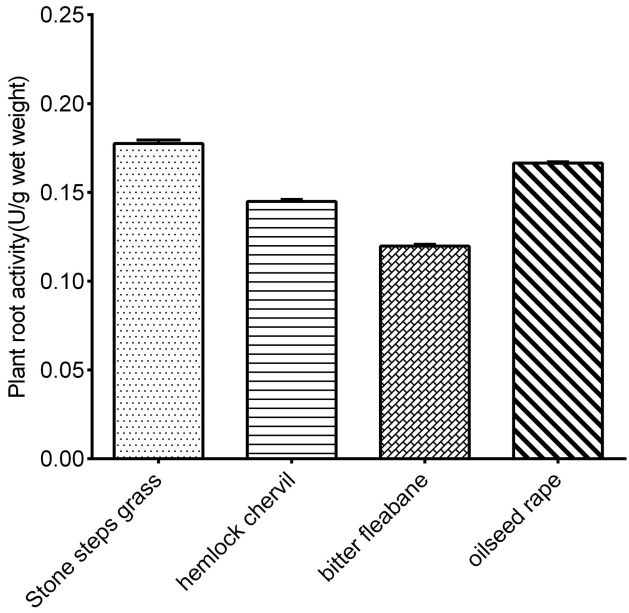
Weigh 0.1-0.3g stone step grass and carry the assay according to the operation steps. The results are as follows:

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 - 0.0162) ÷ 0.0042 ÷ (1000 × 0.2 × 3)

= 0.18 (U/g wet weight)

Detect stone step grass, hemlock chervil, bitter fleabane, oilseed rape according to the protocol, the result is as follows：



Statement

1. This assay kit is for Research Use Only. We will not response for any arising problems or legal responsibilities causing by using the kit for clinical diagnosis or other purpose.
2. Please read the instructions carefully and adjust the instruments before the experiments. Please follow the instructions strictly during the experiments.
3. Protection methods must be taken by wearing lab coat and latex gloves.
4. If the concentration of substance is not within the detection range exactly, an extra dilution or concentration should be taken for the sample.
5. It is recommended to take a pre-test if your sample is not listed in the instruction book.
6. The experimental results are closely related to the situation of reagents, operations, environment and so on. Elabscience will guarantee the quality of the kits only, and NOT be responsible for the sample consumption caused by using the assay kits. It is better to calculate the possible usage of sample and reserve sufficient samples before use.