

Soil Dehydrogenase(S-DHA) Activity Assay Kit

Note: Before the experiment, it is recommended to select 2-3 sample with large expected differences for pre-experiment.

Operation Equipment: Spectrophotometer/Microplate reader

Catalog Number: BC0395

Size: 100T/48S

Product Composition: Before use, please carefully check whether the volume of the reagent is consistent with the volume in the bottle. If you have any questions, please contact Solarbio staff in time.

Components:

Reagent name	Size	Preservation Condition
Reagent I A	Powder×2	2-8°C
Reagent I B	Powder×2	2-8°C
Reagent II	Liquid 15 mL×1	2-8°C
Reagent III	Liquid 30 mL×1 (Required but not provided)	2-8°C

Solution Preparation:

- Reagent I:** Before use, take a bottle of reagent I-B and add it to a bottle of reagent I-A, dissolve it with 2.5 mL distilled water (about 25 S), ready for use. After preparation, it should be stored at 2-8°C away from light. It is best to use it within a week. If it appears red, it should not be used.
- Reagent III:** Ethyl Acetate, self-provided. Provide a 15 mL empty reagent bottle.

Product Description:

The activity of Soil Dehydrogenase (S-DHA) can reflect the active microbial biomass and its degradation activity towards organic matter in the soil system, and can be used as an indicator of soil microbial degradation performance.

The hydrogen receptor 2,3,5-Triphenyl Tetrazolium Chloride (TTC) is reduced to Triphenyl Formazine (TF) during cellular respiration after receiving hydrogen. The TF appears red and has a maximum absorption peak at a wavelength of 485 nm. Its absorbance value is measured at 485 nm to obtain soil dehydrogenase activity.

Required reagents and equipment:

Visible spectrophotometer/Microplate reader, micro glass cuvette/96 well flat-bottom plate (non-polystyrene), mortar, sieve (30-50 meshes), balance, constant temperature incubator/water bath, centrifuge, ice, distilled water, ethyl acetate (>98%, AR).

Procedure:

I. Sample preparation

1. Soil sample: Take 0.05 g of fresh soil sample after 30-50 meshes sieve (To ensure full contact between TTC and soil particles).
2. Mud sample: Wash mud with distilled water, centrifuge at 12000 rpm for 10 minutes at 25°C, discard supernatant, repeat 3- 4 times.

II. Determination operation

1. Preheat Spectrophotometer/microplate reader for 30 minutes, adjust the wavelength to 485 nm, and zero with distilled water.
2. Add the following reagents (add the following reagents in sequence to an EP tube):

Reagent	Control tube (A _C)	Test tube (A _T)
Sample (g)	0.05	0.05
Reagent I (mL)	-	0.1
Reagent II (mL)	0.2	0.1
Mix thoroughly in centrifuge tube, incubate at 37°C dark place for 24 hours, then keep on ice for 5 minutes immediately.		
Reagent III (mL)	0.3	0.3
<p>Shake vigorously for 10 minutes, 15000rpm, 4 °C, centrifuge for 10 minutes, take 200 μL of the supernatant in micro glass cuvette or 96-well plate (non polystyrene material), measure the absorbance of the control tube and the test tube at 485 nm, and record them as A_C and A_T, respectively. Calculate $\Delta A = A_T - A_C$.</p> <p>Note: One test tube is required for one control tube.</p>		

III. Calculation:

A. Micro glass cuvette

Definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the increasing absorbance of every 0.01 for per hour in per milliliter reaction system at 37°C every gram of sample.

$$s\text{-DHA (U/g)} = \Delta A \div 0.01 \div T \div W \times V_{rv} = 41.67 \times \Delta A$$

B. 96 well flat-bottom plate

Definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the increasing absorbance of every 0.005 for per hour in per milliliter reaction system at 37°C every gram of sample.

$$s\text{-DHA (U/g)} = (U/g) = \Delta A \div 0.005 \div T \div W \times V_{rv} = 83.33 \times \Delta A$$

T: Incubation time, 24 hours

W: Sample weight, 0.05 g;

V_{rv}: Total reaction volume, 0.5 mL.

Note:

1. Reagent III is volatile and toxic, please wear lab clothes, masks and latex gloves for your health.

2. If the absorbance is larger, detect again after decreasing the sample. If the absorbance value is too small, the culture time can be appropriately prolonged.
3. If the supernatant to be tested remains turbid after centrifugation, try increasing the centrifugation speed or extending the time, such as 15000rpm, 4 °C, centrifugation for 20 minutes.

Recent Product Citations:

[1] Huang J, Ye J, Gao W, Liu C, Price GW, Li Y, Wang Y. Tea biochar-immobilized *Ralstonia Bcul-1* increases nitrate nitrogen content and reduces the bioavailability of cadmium and chromium in a fertilized vegetable soil. *Sci Total Environ.* 2023 Mar 25;866:161381. doi: 10.1016/j.scitotenv.2022.161381. Epub 2023 Jan 5. PMID: 36621509.

[2] Gong P, Gao D, Hu X, Tan J, Wu L, Liu W, Yang Y, Jin E. Changes of bacterial and fungal communities and relationship between keystone taxon and physicochemical factors during dairy manure ectopic fermentation. *PLoS One.* 2022 Dec 19;17(12): e0276920. doi: 10.1371/journal.pone.0276920. PMID: 36534655; PMCID: PMC9762577.

[3] Li N, Chen J, Liu C, Yang J, Zhu C, Li H. Cu and Zn exert a greater influence on antibiotic resistance and its transfer than doxycycline in agricultural soils. *J Hazard Mater.* 2022 Feb 5;423(Pt B):127042. doi: 10.1016/j.jhazmat.2021.127042. Epub 2021 Aug 26. PMID: 34536850.

[4] Liu T, Wang S, Chen Y, Luo J, Hao B, Zhang Z, Yang B, Guo W. Bio-organic fertilizer promoted phytoremediation using native plant *leymus chinensis* in heavy Metal(loid)s contaminated saline soil. *Environ Pollut.* 2023 Jun 15; 327:121599. doi: 10.1016/j.envpol.2023.121599. Epub 2023 Apr 8. PMID: 37037280.

[5] Zhuang X, Wang Y, Wang H, Dong Y, Li X, Wang S, Fan H, Wu S. Comparison of the efficiency and microbial mechanisms of chemical- and bio-surfactants in remediation of petroleum hydrocarbon. *Environ Pollut.* 2022 Dec 1;

References:

[1] Kumar S, Chaudhuri S, Maiti S K. Soil dehydrogenase enzyme activity in natural and mine soil-a review[J]. *Middle-East Journal of Scientific Research*, 2013, 13(7): 898-906.

[2] Friedel J K, Mölter K, Fischer W R. Comparison and improvement of methods for determining soil dehydrogenase activity by using triphenyltetrazolium chloride and iodonitrotetrazolium chloride[J]. *Biology and fertility of soils*, 1994, 18(4): 291-296.

Related Products:

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|---------------|---|
| BC0860/BC0865 | Soil Acid Protease Activity Assay Kit |
| BC0280/BC0285 | Soil Alkaline Phosphatase(S-AKP/ALP) Activity Assay Kit |
| BC0110/BC0115 | Soil Polyphenoloxidase Activity Assay Kit |

BC4030/BC4035 Soil β -1,4-Glucanase Activity Assay Kit

