



CheKine™ Micro Soil Aryl Sulfatase (S-ASF) Activity Assay Kit

Cat #: KTB4054

Size: 48 T/48 S 96 T/96 S

	Micro Soil Aryl Sulfatase (S-ASF) Activity Assay Kit		
REF	Cat #: KTB4054	LOT	Lot #: Refer to product label
	Applicable sample: Soil sample		
	Storage: Stored at -20°C for 6 months, protected from light		

Assay Principle

Soil arylsulfatase originates from soil microorganisms and facilitates the conversion of organic sulfur compounds in the soil into inorganic forms that can be absorbed by plants. It plays a crucial role in the biochemical cycle of sulfur and plant sulfur nutrition metabolism, serving as an important biological indicator of soil quality. CheKine™ Micro Soil Aryl Sulfatase (S-ASF) Activity Assay Kit provides a simple, convenient, and rapid method for detecting S-ASF activity in soil samples. The principle behind this assay is that S-ASF catalyzes the hydrolysis of potassium p-nitrobenzenesulfate to produce p-nitrophenol, which has a characteristic absorption peak at 410 nm.

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	48 T	96 T	
Reagent I	26 mL	52 mL	4°C
Reagent II	Powder×1 vial	Powder×2 vials	-20°C, protected from light
Reagent III	6.5 mL	13 mL	4°C
Reagent IV	26 mL	52 mL	4°C
Standard	1 mL	1 mL	4°C, protected from light

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

Materials Required but Not Supplied

- Microplate reader or visible spectrophotometer capable of measuring absorbance at 410 nm
- 96-well plate or microglass cuvette, precision pipettes, disposable pipette tips
- Oven, 30-50 mesh sieve, centrifuge, constant temperature water bath, analytical balance
- Deionized water, toluene

Reagent Preparation

Reagent I: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4°C.

Working Reagent II: Prepared before use. According to the dosage, take one vial of Reagent II and add 3.5 mL of deionized water, dissolve thoroughly and set aside for use; any unused reagent should be aliquoted and stored at 20°C in the dark for up to one week. Avoid repeated freeze-thaw cycles.

Reagent III: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4°C.

Reagent IV: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4°C.

Standard: 5 mM p-nitrophenol standard solution. Ready to use as supplied. Equilibrate to room temperature before use. Store at 4°C, protected from light.

Note: Reagent II and Standard are toxic, so it is recommended to experiment in a fume hood.

Standard preparation: Use 5 mM p-nitrophenol standard solution, prepare standard curve dilution as described in the table.

Num.	Standard Volume	Deionized Water Volume (μL)	Concentration (mM)
Std.1	100 μL 5 mM Standard	900	0.5
Std.2	500 μL of Std.1 (0.5 mM)	500	0.25
Std.3	500 μL of Std.2 (0.25 mM)	500	0.125
Std.4	500 μL of Std.3 (0.125 mM)	500	0.0625
Std.5	500 μL of Std.4 (0.0625 mM)	500	0.03125
Std.6	500 μL of Std.5 (0.03125 mM)	500	0.01563
Blank	0	500	0 (Blank Tube)

Notes: Always prepare fresh standards per use; Diluted Standard Solution is unstable and must be used within 4 h.

Sample Preparation

Note: Fresh samples are recommended.

Fresh soil samples should be air-dried naturally or dried in an oven at 37°C, then passed through a 30-50 mesh sieve.

Assay Procedure

1. Preheat the microplate reader or visible spectrophotometer for more than 30 min, and adjust the wavelength to 410 nm, visible spectrophotometer was returned to zero with deionized water.

2. Operation table (The following operations are operated in the 1.5 mL EP tube):

Reagent	Test Tube (μL)	Control Tube (μL)	Standard Tube(μL)
Air-dried soil sample (g)	0.05	0.05	0
Toluene	12.5	12.5	0

Shake to mix thoroughly, ensuring all the soil is moistened, and then let it sit at room temperature for 15 min

Reagent I	200	200	0
Reagent II	50	0	0
Deionized water	0	50	0

Mix thoroughly, then incubate in a 37°C water bath for 1 h

Standard	0	0	262.5
Reagent III	50	50	50

Reagent IV	200	200	200
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Mix thoroughly and keep at room temperature for 2 min, then centrifuge at 10,000 g for 10 min at 25°C, transfer 200 µL of the supernatant to a 96-well plate or a micro glass cuvette. Measure the absorbance at 410 nm. Record the absorbance values as A_{Test} , A_{Control} , A_{Standard} , and A_{Blank} , respectively. Calculate $\Delta A_{\text{Test}} = A_{\text{Test}} - A_{\text{Control}}$, $\Delta A_{\text{Standard}} = A_{\text{Standard}} - A_{\text{Blank}}$.

Note: The standard curve needs to be determined only once, and a control well should be set up for each measurement well. Before the experiment, it is suggested that 2-3 samples with large expected differences should be selected for pre-experiment. If ΔA_{Test} is less than 0.1, the sample volume can be appropriately increased, and the calculation formula should be adjusted accordingly. If ΔA_{Test} is greater than $\Delta A_{\text{Standard}}$ of 0.5 mM, the sample supernatant can be further diluted by deionized water, and the calculation result should be multiplied by the dilution multiple.

Data Analysis

Note: We provide you with calculation formulae, including the derivation process and final formula. The two are exactly equal. It is suggested that the concise calculation formula in bold is final formula.

1. Drawing of standard curve:

With the concentration of the standard solution as the x-axis and the $\Delta A_{\text{Standard}}$ as the y-axis, draw the standard curve, get the standard equation, and bring the ΔA_{Test} into the equation to get the x value (mM).

2. Calculation of S-ASF activity:

Active unit definition: One unit of enzyme activity is defined as the production of 1 µmol of p-nitrophenol per g of soil sample per day.

$$\text{S-ASF (U/g soil sample)} = x \times V_{\text{Total reaction}} \div W \div T = \mathbf{6.3 \times x \div W}$$

Where: T: Reaction time, 1 h=1/24 d; $V_{\text{Total reaction}}$: Total volume of reaction system, 0.2625 mL; W: Sample mass, 0.05 g.

Typical Data

Typical standard curve:

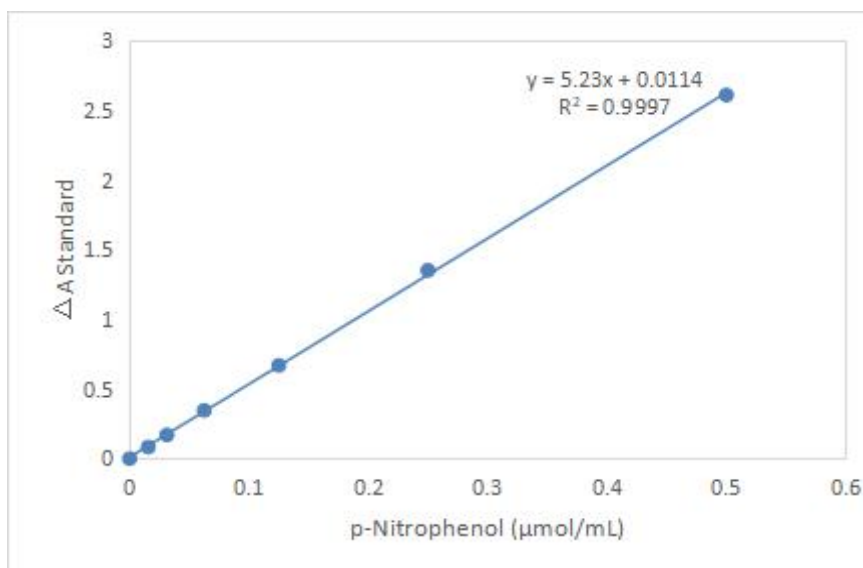


Figure1. p-Nitrophenol standard curve

Examples:

Take 0.05 g of fresh soil sample that has been dried in a 37°C oven and use 96-well plate to calculate $\Delta A_{\text{Test}} = 0.498 - 0.08 = 0.418$, $x = 0.078$. The content calculated according to the soil sample mass is as follows:

$$\text{S-ASF (U/g soil sample)} = 6.3 \times 0.078 \div 0.05 = 9.828 \text{ U/g.}$$

Recommended Products

Catalog No.	Product Name
KTB4023	CheKine™ Mirco Soil Peroxidase (S-POD) Activity Assay Kit
KTB4024	CheKine™ Mirco Soil Acid Protease (S-ACPT) Activity Assay Kit
KTB4025	CheKine™ Mirco Soil β -Xylosidase (S- β -XYS) Activity Assay Kit

Disclaimer

The reagent is only used in the field of scientific research, not suitable for clinical diagnosis or other purposes. For your safety and health, please wear a lab coat and disposable gloves.