# EZMeta™ Soil Amylase (S-AL) Colorimetric Activity Kit

Cat #: D-AKC4030

Size: 48T / 96T

Storage: Stored at 4°C for 12 months, protected from light

## Product Information

**Applicable samples:** Soil sample

## Assay Principle

Amylase (EC3.2.1.1) is a general term for a class of enzymes that catalyze starch hydrolysis. Amylase in soil mainly comes from microorganisms. Amylase hydrolyzes starch to produce reducing sugar, which can react with 3,5-Dinitrosalicylic acid to produce reddish brown substances. There is a characteristic absorption peak at 540 nm, and the color depth is proportional to the amount of reducing sugar within a certain range.

## Materials Supplied and Storage Conditions

| Kit components | Size | | Storage conditions |
|---|---|---|---|
| | 48 T | 96 T | |
| Reagent Ⅰ | 10 mL | 20 mL | 4°C |
| Reagent Ⅱ | 4 mL | 8 mL | 4°C |
| ReagentⅢ | 12 mL | 24 mL | 4°C |
| Standard | 1 | 1 | 4°C, protected from light |

## Materials Required but Not Supplied

·Microplate reader or visible spectrophotometer capable of measuring absorbance at 540 nm

·96-well plate or microglass cuvette, precision pipettes, disposable pipette tips

·Thermostat water bath, constant temperature shaker, centrifuge, 30-50 mesh sieve

·Deionized water, toluene

## Reagent Preparation

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

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

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

**Standard preparation:**

**Standard:** Prepare before use, add 1 mL deionized water to prepare 10 mg/mL glucose standard solution; Store at 4°C, protected from light. Prepare standard curve dilution as described in the table:

| Num. | 10 mg/mL Standard (μL) | Deionized water (μL) | Concentration (mg/mL) |
|---|---|---|---|
| Std.1 | 40 | 160 | 2 |
| Std.2 | 20 | 180 | 1 |
| Std.3 | 10 | 190 | 0.5 |
| Std.4 | 5 | 195 | 0.25 |
| Std.5 | 2.5 | 197.5 | 0.125 |
| Std.6 | 1.25 | 198.75 | 0.063 |
| Blank | 0 | 200 | 0 |

## Sample Preparation

Naturally dried fresh soil samples or air drying in oven at 37°C, pass through a 30-50 mesh sieve.

**Note: Fresh samples are recommended. If the experiment is not carried out immediately, the samples can be stored at -80°C for several weeks. During the determination, the temperature and time of thawing should be controlled. When thawing at room temperature, the sample should be thawed within 4 h.**

## Assay Procedure

1. Preheat the microplate reader or visible spectrophotometer for more than 30 min, and adjust the wavelength to 540 nm, visible spectrophotometer was returned to zero with deionized water.

2. Operation table (the following operations are performed in 1.5 mL EP tubes):

| Reagent | Control Well (μL) | Test Well (μL) | Standard Well (μL) | Blank Well (μL) |
|---|---|---|---|---|
| Air dried soil sample (g) | 0.04 | 0.04 | 0 | 0 |
| Toluene | 20 | 20 | 0 | 0 |
| Keep in 25°C for 15 min | | | | |
| Reagent Ⅰ | 100 | 100 | 0 | 0 |
| Reagent Ⅱ | 0 | 120 | 0 | 0 |
| Deionized water | 120 | 0 | 0 | 0 |
| Mix well, shake at 37°C for 24 h, 25°C, 8,000 rpm, centrifuge for 10 min, and take the supernatant | | | | |
| Supernatant | 120 | 120 | 0 | 0 |
| Standard | 0 | 0 | 120 | 0 |
| Deionized water | 0 | 0 | 0 | 120 |
| ReagentⅢ | 120 | 120 | 120 | 120 |

3. Mix well, keep in 90°C water bath for 5 min, and after cooling, take 200 μL to determine the 540 nm absorbance value in a microglass cuvette or 96 well plate. The absorbance of control well, test well, standard well, blank well, were recorded as $A_{Control}$, $A_{Test}$, $A_{Standard}$ and $A_{Blank}$. Finally, calculate $\Delta A_{Test}=A_{Test}-A_{Control}$, $\Delta A_{Standard}=A_{Standard}-A_{Blank}$.

**Note: 1. Blank well and standard well only need to measure 1 time. Control well is necessary for each test. In order to guarantee the accuracy of experimental results, pre-experiments are suggested for 2-3 samples with potential significant difference. 2. Keep the same cooling time after 90°C water bath is the same for each experiment, and the absorbance value should be determined within 30 min.**

## 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 $\Delta A_{Standard}$ as the y-axis, draw the standard curve, get the standard equation y=kx+b, and bring the $\Delta A_{Test}$ into the equation to get the x value (mg/mL).

2. Calculation of enzyme activity:

Definition of unit: 1 mg of reducing sugar per g of soil sample per day is defined as one unit of soil amylase activity.

S-AL(U/g soil sample)=x×$V_{Total\ volume}$÷W÷T=**6×x**

$V_{Total\ volume}$: total volume of enzymatic reaction, 0.24 mL; W: mass of soil sample, 0.04 g; T: reaction time, 1 d.

## Typical Data

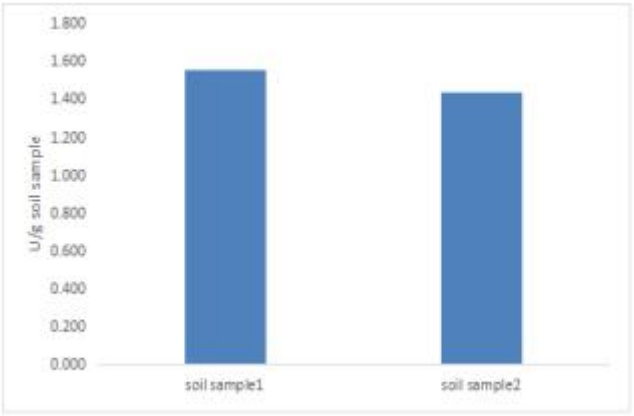


Figure 1. Determination of amylase activity in soil by this assay kit

## Disclaimer

The reagent is only used in the field of scientific research, not suitable for clinical diagnosis or other purposes.