# Soil β-1,4-Glucanase/ Cellobiosidase (S-C1) Activity Assay Kit

**Note:** Take two or three different samples for prediction before test.

**Operation Equipment:** Spectrophotometer/ Microplate reader

**Catalog Number:** BC4035

**Size:** 100T/48S

## Components:

**Reagent I:** Toluene 2mL×1. Storage at 4℃. **(self-provided reagent)**

**Reagent II:** Powder×2. Storage at 4℃. Add 7.5 mL of Reagent III to fully dissolve when the solution will be used. The left reagent can be stored at 4°C for one weeks.

**Reagent III:** 40 mL×1. Storage at 4℃.

**Reagent IV:** 30 mL×1. Storage at 4℃.

**Standard solution:** 1mL×1, 5mmol/L p-nitrophenol solution. The standard is diluted 50 times with reagent III to obtain a 100 μmol/L standard solution.

## Product Description

β-1,4-glucanase/cellobiosidase (C1, EC3.2.1.91) exists in bacteria, fungi and animals, and is a component of the cellulase system. The end of the linear molecule hydrolyzes the β-glucosidic bond and cuts out one cellobiose molecule every time.

S-C1 can catalyze p-nitrobenzene cellobiose (PNPC) to p-nitrophenol, which has a characteristic light absorption at 400nm.

## Reagents and Equipment Required but Not Provided

Spectrophotometer/ Microplate reader, centrifuge, water-bath/ Constant temperature incubator, transferpettor, micro glass cuvette/ 96-well plate, mortar, **toluene,** sieve (30-50 mesh) and distilled water.

## Procedure

### 1. Sample preparation:

Fresh soil samples are naturally air-dried or oven dried at 37℃ and passed through a 30-50 mesh sieve.

### 2. Determination steps and sample adding table:

a. Preheat spectrophotometer/microplate reader more than 30 min, adjust wavelength to 400 nm and spectrophotometer set zero with distilled water.

b. Operate according to the following table:

| Reagent Name | Test tube (T) | Control tube (C) | Standard tube (S) | Blank tube (B) |
|---|---|---|---|---|
| Soil sample (g) | 0.03 | 0.03 | | |
| Reagent I | 15 | 15 | | |
| Mix by shaking to make the soil sample wet and leave it for 15min at room temperature | | | | |

| | | | | |
|---|---|---|---|---|
| Reagent II (μL) | 120 | | | |
| Reagent III (μL) | 150 | 150 | | |
| Mix well. After reacting for 1 h at 37°C in a water bath, immediately boil in a water bath for 5 min (close tightly to prevent water loss) and cool in running water/ice bath. | | | | |
| Reagent II | | 120 | | |
| Centrifuge at 10,000 rpm and 25℃ for 10 min and take the supernatant | | | | |
| Supernatant | 100 | 100 | | |
| Standard solution (μL) | - | - | 100 | |
| Distilled water | | | | 100 |
| Reagent IV (μL) | 200 | 200 | 200 | 200 |

Mix well, react for 2 minutes at RT. Pipette 200μL into a micro glass cuvette /96-well plate and measure the absorbance A at 400nm, calculate $\Delta A = A_T - A_C$, $\Delta A_S = A_S - A_B$

**Calculation of S-C1 activity:**

1. Calculation of S-C1activity:

Unit definition: Unit definition: one unit is defined as the amount of enzyme that catalyzes the production of 1 μmol of p-nitrophenol per day every gram of soil catalyzes at 37°C.

S-C1 activity (U/mg) $=\Delta A \div (\Delta A_S \div C_S) \times V1 \div W \div T = 0.684 \times \Delta A \div \Delta A_S \div W$

$C_S$：concentration of standard solution，100μmol/L

V1: the volume of reaction system, $2.85\times10^{-4}$ L;

W: sample fresh weight, g;

T: reaction time: 1/24d.

**Note**

1. If the absorbance value is greater than 1.5, it is recommended to dilute the supernatant with reagent III and determine with decrease the quality of soil samples.

**Experimental Examples:**

1. Take two tubes of 0.03g soil sample, which are the measuring tube and the control tube. Follow the measuring steps and mark them as At and Ac. Measure with 96-well plate and calculate $\Delta A = A_T - A_C = 0.444-0.244=0.2$, $\Delta A_S = A_S - A_B = 0.413-0.048=0.365$, calculate the enzyme activity:
   S-C1 activity (U/g soil) ）$=0.684\times \Delta At \div \Delta A_S \div W = 0.684\times0.2\div0.365\div0.03=12.493$ U/g soil.
2. Take two tubes of 0.03g forest soil samples, which are the measuring tube and the control tube. Follow the measuring steps and mark them as At and Ac. Measure with 96-well plate and calculate $\Delta A = A_T - A_C = 0.33-0.195=0.135$, $\Delta A_S = A_S - A_B = 0.413-0.048=0.365$, calculate the enzyme activity:

S-C1 activity (U/g soil ) =0.684×ΔAt÷ΔAs÷W=0.684×0.1135÷0.365÷0.03=8.4329 U/g soil

**Related Products:**

BC4010/BC4015 Soil β-Xylosidase (S-β-XYS) Activity Assay Kit
BC3080/BC3085 Soil α-glucosidase (S-α-GC) Activity Assay Kit
BC0240/BC0245 Soil Saccharase (S-SC) Activity Assay Kit