SunLong Biotech Co.,LTD
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Cellulase (CL) Assay Kit

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

Operation Equipment: Spectrophotometer/ microplate reader

Catalog Number: AK0210

Size:100T/48S

Components:

Extract reagent: 100mL×1, storage at 4℃ .

Reagent 1: 5mL×1, storage at 4℃ .

Reagent 2: 20mL×1, storage at 4℃ .

Reagent 3: 5mL×1, storage at 4℃ .

Standard: powder ×1, storage at 4℃ . 10mg of anhydrous glucose (Loss on drying<0.2%), add 1mL of distilled water to dissolve before use, prepare a 10mg / mL glucose solution for future use, and store at 4 ℃ for 1 wee.

Prepared standard: The 10mg/mL standard solution dilute to 1, 0.8, 0.6, 0.4, 0.2, 0. 1, 0 mg/mL for use.

Product Description:

Cellulase (EC 3.2. 1.4) exists in bacteria, fungi and animals， which can catalyze cellulose degradation. It is a type of enzyme preparation that can be widely used in the fields of medicine, food, cotton spinning, environmental protection and renewable resource utilization.

The 3.5-dinitrosalicylic acid method is used to determine the reducing sugar content of cellulose catalyzed by CL.

Reagents and Equipment Required but Not Provided:

Spectrophotometer/ microplate reader, adjustable transferpettor, balance, mortar/homogenizer, centrifuge, micro glass cuvette/ 96 well flat-bottom plate, ice and distilled water.

Sample preparation:

1. Plant and animal tissues: Weigh about 0.1 g of sample, add 1 mL of Extract reagent and fully grind. Centrifugate at 8000g and 4℃ for 10 min, the supernatants as samples to be tested.
2. Bacteria or cells: Collect the bacteria or cells into a centrifuge tube, discard the supernatant after centrifugation; add 1 mL of Extract reagent for every 5 million bacteria or cells, and break the bacteria or cells with an ultrasonic ice bath (power 20%, ultrasonic 3 seconds, interval 10 seconds, repeat 30 times); Centrifugate at 8000g and 4℃ for 10 min, take the supernatant and place on ice for testing.

Procedure:

1. Preheat spectrophotometer/ microplate reader for 30min, adjust the wavelength to 540 nm and set the counter to zero with distilled water.
2. Add reagent to a 1.5mL EP tube:

Reagent name （μL）	Control tube (Ac)	Test tube (At)	Standard tube (As)
Reagent 1	50	50	-

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Reagent 2	200	200	-
Distilled water	50	50	-
Sample		50	-
Boiled sample	50		-
Mix well, and react accurately in water bath at 40℃ for 30min. after taking out, put it in boiling water and boil for 15 min immediately to get the saccharification solution.			
Saccharification solution	15	15	-
Standard solution	-	-	15
Reagent 3	35	35	35
Mix well, boil for 15min in a boiling water bath and cool.			
Distilled water	250	250	250

Mix well, set the counter to zero with distilled water, and measure the absorbance A at 540 nm, and calculate $\Delta A = A_T - A_C$.

Calculation:

1. set the counter to zero with the standard tube 0mg/mL at 540 nm and read the absorbance value a of the standard tube. According to concentration of standard solution and absorbance to create the standard curve, take standard solution as Y-axis, ΔAs as X-axis. Take ΔA into the equation to obtain y (mg/mL).
2. **Protein concentration:**

Unit definition: One unit of enzyme activity is defined as the amount of enzyme that catalyzes the production of 1μg glucose per minute in the reaction system every milligram tissue protein

CL (U/mg prot) = 1000 ×y ×Vrv÷ （Vs ×Cpr） ÷T = 233y÷Cpr

3. **Sample weight:**

Unit definition: One unit of enzyme activity is defined as that one gram tissue catalyzes the production of 1μg glucose per min in the reaction system.

CL (U/g) =1000× y×Vrv÷ （Vs×W÷Ve） ÷T = 233y÷W

4. **Cells or bacteria:**

Unit definition: One unit of enzyme activity is defined as that 10^4 cells or bacteria catalyzes the production of 1μg glucose in the reaction system per min.

CL (U/10^4 cell) = 1000× y×Vrv÷(500×Vs÷Ve) ÷T=0.467×y

1000: 1mg/mL = 1000μg/mL

Vrv: Total volume of reaction system, 0.35mL.

Vs: sample volume added, 0.05mL;

Ve: volume used in the extraction solution, 1mL;

Cpr: sample protein concentration, mg/mL;

W: Fresh weight of sample, g;

T: React time, 30min.

500: the number of cells or bacteria, 500×10 thousand.

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Recent Product Citations:

Guo Q, Du G, Qi H, et al. A nematicidal tannin from Punica granatum L. rind and its physiological effect on pine wood nematode (Bursaphelenchus xylophilus)[J]. Pesticide biochemistry and physiology, 2017, 135: 64-68.

References:

Faria M L, Kolling D, Camassola M, et al. Comparison of Pennicillium echinulatum and Trichoderma reesei cellulases in relation to their activity against various cellulosic substrates[J]. Biores. Technol, 2008, 99: 1417- 1424.

Related Products:

AK0291/AK0290	Glucogen Content Assay Kit
AK0229/AK0227	Plant Tissue Fructose Content Assay Kit
AK0218/AK0217	Trehalase Activity Assay Kit