Cellulases

Structure:
The precise properties of cellulases vary depending on their origin. The majority of microbial cellulases studied have been shown to be acidic proteins with a significant carbohydrate content [2,3].

Specificity:
Cellulase preparations are able to decompose natural cellulose(e.g. filter paper) as well as modified celluloses such as carboxymethyl cellulose or hydroxyethyl cellulose. Cellulase hydrolyses 1,4-b-D-glucosidic linkages in cellulose, lichenin and cereal b-D-glucans. The exoglucanases are thought to act primarily on newly generated chain ends producing mainly cellobiose [4]. b-Glucosidase hydrolyses terminal b-D-glucose residues from the ends of cellulose molecules. In nature, cellulose is found in association with other components e.g. hemicellulose, lignin and pectin. SERVA cellulases contain a number of other activities which assist in breaking down these components and degrading cell walls. a-Amylase hydrolyses 1,4-a-D-glucosidic linkages in polysaccharides containing three or more 1,4-a-linked D-glucose units. Pectinaserandomly cleaves 1,4-a-D-galactosiduronic linkages in galacturans. These products also contain hemicellulase and protease activities.

Physical and Chemical Properties:
Most cellulases studied have similar pH optima, solubility and amino acid composition. Thermal stability and exact substrate specificity may vary. However, it should be remembered that cellulase preparations generally contain other enzymatic activites besides cellulase, and these may also affect the properties of the preparations.

Optimum pH: Cellulase preparations are effective between pH 3 and 7. The optimum pH generally lies between 4 and 5.
Optimum temperature: 40 - 50 ℃

Inhibitors:
Cellulase is inhibited by its reaction products e.g. glucose, cellobiose. Hg inhibits cellulases completely, whereas Mn, Ag,Cu and Zn ions are only slightly inhibitory.

Stability and storage:
The activity of cellulase preparations has been found to be completely destroyed after 10-15 minutes at 80 ℃. Solutionsof cellulase at pH 5-7 are stable f or 24 hours at 4 ℃. These products should be stored at 4 ℃, in a dry place in tightlyclosed containers. I f stored in this manner, lyophilized preparation are stable for several months without significant loss of activity.

Applications:
Cellulase is used extensively in the isolation of plant protoplasts, frequently in combination with Macerozyme R10 (cat.no. 28302). Protoplasts are essentially plant cells from which the cell walls have been removed. They are used in plant virus studies, metabolic investigations and genetic modification experiments [5,6,7,8].

Assay methods and unit definitions:
Cellulase liberates glucose from carboxymethyl cellulose which is determined colourimetrically with alkaline copper reagent [9]. 1 U catalyses the liberation of 1 µmole glucose from sodium carboxymethyl cellulose per minute at 40 ℃, pH 4.5.

a-amylase is assayed by its ability to produce reducing groups from starch, which are measured by the reduction of 3,5-dinitrosalicylic acid [10]. 1 U catalyzes the liberation of 1 microequivalent of reducing groups from soluble starch per minute at 25 ℃, pH 6.0, calculated as maltose.

Pectinase catalyzes the hydrolysis of pectic acid; liberated D- galacturonic acid is determined with alkaline copper reagent[11]. 1 U catalyzes the liberation of 1 microequivalent of reducing groups from pectic acid per minute at 25 ℃, pH 4.5 calculated as galacturo nic acid.

Protease activity is determined by the hydrolysis of dimethylcasein, liberated amino acids being determined with 2,4,6-trinitrobenzene sulfonic acid [12].1 DMC-U catalyzes the cleavage of 1 microequivalent peptide bond from dimethyl casein per minute at 25 ℃, pH 7.0 expressed in terms of newly formed terminal amino groups.

Hemicellulase catalyzes the hydrolysis of xylan from oat spelts, and the reducing groups liberated are determined with alkaline copper reagent [9].1 U catalyzes the liberation of 1 microequivalent reducing groups from xylan per hour at 37 ℃, pH 5.5 calculated as xylose.

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Product Name	Cat.No.*
Cellulase Onozuka R-10 from *Trichoderma viride* ca. 1 U/mg	16419
Cellulase Onozuka RS from *Trichoderma viride* ca. 2 U/mg	16420
Cellulase from *Trichoderma viride* ca. 1.5 U/mg	16426