**DESCRIPTION**

**ACCELLERASE® 1500** cellulase is an enzyme complex intended specifically for the lignocellulosic biomass processing industries, including renewable fuels and chemicals. **ACCELLERASE® 1500** is a significant step toward the more cost-effective commercial scale production of cellulosic ethanol and will facilitate process development and scale up in this emerging industry. Key features that are expected to be important at commercial scale biorefineries are already built in to this product. Benefits observed with the proprietary enzyme complex and the unique product formulation of **ACCELLERASE® 1500** compared to conventional cellulases include:

- Enhanced saccharification performance on a variety of feedstocks.
- Ability to operate in simultaneous saccharification and fermentation (SSF) processes, two step sequential hydrolysis and fermentation (SHF) processes, or hybrid saccharification and fermentation (HSF) processes.
- High beta-glucosidase activity to minimize residual cellobiose, which may lead to higher rates of saccharification and ultimately to a faster ethanol fermentation. Yields may also be improved.
- Whole broth product. The remaining nutrients from enzyme production are available to the yeas. This may lead to faster ethanol fermentations, reduce the cost of ethanol fermentation raw materials and possibly even improve ethanol yields.
- Improved formulation to reduce risk of inhibition of fermentative organisms, especially at high solids processing. The formulation does not interfere with saccharification carbohydrate profile analysis.

**ACCELLERASE® 1500** enzyme complex contains a potent combination of enzymes which effectively modify and digest non-starch carbohydrates, the structural material of lignocellulosic biomass. Lignocellulosic material is composed mainly of cellulose, hemicellulose and beta-glucans, which are associated with each other and also with lignin, pectins, proteins, starch and lipids. **ACCELLERASE® 1500** is capable of efficiently and synergistically hydrolyzing lignocellulosic biomass into fermentable monosaccharides. **ACCELLERASE® 1500** contains high levels of beta-glucosidase activity to ensure almost complete conversion of cellobiose to glucose.
ACCELLERASE® 1500 is produced with a genetically modified strain of Trichoderma reesei. The production host is inactivated at the end of the controlled fermentation.

**TYPICAL CHARACTERISTICS**

ACCELLERASE® 1500 enzyme complex contains multiple enzyme activities: exoglucanase, endoglucanase, hemi-cellulase, and beta-glucosidase and others. The endoglucanase activity is standardized on the basis of its activity on carboxymethylcellulose (CMC). Beta-glucosidase activity is standardized on the basis of activity on pNP-glucoside. The biomass hydrolysis performance of this enzyme complex is a result of the synergistic effect of all the main and accessory activities and cannot be completely evaluated on the basis of the declared activities alone.

<table>
<thead>
<tr>
<th>Enzyme Activity</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Endoglucanase Activity</td>
<td>2200 – 2800 CMC U/g</td>
</tr>
<tr>
<td>Beta-Glucosidase Activity</td>
<td>450 – 775 pNPG U/g</td>
</tr>
<tr>
<td>Appearance</td>
<td>Brown liquid</td>
</tr>
<tr>
<td>pH</td>
<td>4.6 – 5.0</td>
</tr>
</tbody>
</table>

The activity of ACCELLERASE® 1500 enzyme complex is expressed in carboxymethylcellulose (CMC U) activity units. One CMC U unit of activity liberates 1 µmol of reducing sugars (expressed as glucose equivalents) in one minute under specific assay conditions of 50°C (122°F) and pH 4.8. Beta-glucosidase is reported in pNPG units. One pNPG unit denotes 1 µmol of Nitrophenol liberated from para-nitrophenyl-B-D-glucopyranoside per minute at 50°C (122°F) and pH 4.8. Detailed assay methods are available upon request.

**APPLICATION RECOMMENDATIONS**

ACCELLERASE® 1500 enzyme complex will hydrolyze the lignocellulosic carbohydrates into fermentable monosaccharides and as aid materials handling by liquefaction and viscosity reduction. Feedstocks including paper pulp, corn stover and cob, sugar cane bagasse, wheat straw, wood chips, waste paper and many others can all be hydrolyzed using ACCELLERASE® 1500. ACCELLERASE® 1500 can work with a variety of pretreatments including dilute acid, ammonia fiber-expansion (AFEX) and steam expansion. This can be done using simultaneous saccharification and fermentation (SSF), in a two step sequential hydrolysis and fermentation (SHF), or hybrid saccharification and fermentation (HSF) configuration. Please be aware that the pH and temperature stability optima and limits of the enzyme in use will depend upon the other operating parameters and your process configuration. Saccharification performance may be enhanced by the addition of other DuPont® enzymes depending on the composition of the pretreated feedstock.

**DOSAGE GUIDELINES**

The optimum dosage levels of ACCELLERASE® 1500 enzyme complex will vary considerably with different substrates and their associated pretreatment technologies and conditions. Operating conditions such as pH, temperature and reaction time may also affect enzyme performance. An ACCELLERASE® 1500 dosage rate of 0.1 – 0.5 mL per gram cellulose or roughly 0.05 to 0.25 mL per gram of biomass (depending on biomass composition) is recommended as a starting point for optimization of enzyme dosage. ACCELLERASE® 1500 rapidly liquefies and hydrolyzes a variety of substrates within 24 hours, with some additional benefit by extending the time. Small-scale experiments are recommended to determine optimum enzyme performance and dosage in each system. Figures 1 and 2 show the results of using ACCELLERASE® 1500 enzyme on two very different feedstocks in both sacharification only and SSF process configurations.
**Figure 1:** Percent glucan conversion of a pretreated softwood pulp*, and a washed acid-pretreated bagasse (APB) using ACCELLERASE® 1500 enzyme at 0.24 mL/g cellulose, 7% cellulose loading, 50°C, and pH 5.0. 100 g batch-scale experiments in 500ml shake-flasks.

**Figure 2:** Simultaneous saccharification and fermentation (SSF) of a pretreated softwood pulp* and a washed acid-pretreated sugarcane bagasse (APB) using ACCELLERASE® 1500 enzyme at 0.24 mL/g cellulose, 7% cellulose loading, pH 5.0, and 38°C with THERMOSACC® DRY yeast (Milwaukee, WI), 100 g batch-scale experiments in 500 mL shake-flasks.

*Data on pretreated softwood pulp was generated in research funded by Agence Nationale de la Recherche, France (ARN-05-BIOE-007) through L’Agence de l’Environnement et de la Maîtrise de l’Energie (ADEME 0501 C0099).

**PACKAGING**

ACCELLERASE® 1500 enzyme complex is available in various package sizes. Please consult your DuPont® representative for detailed information.

**STORAGE**

It is advisable to store ACCELLERASE® 1500 enzyme complex under refrigerated conditions of 4°C (39°F) and sheltered against direct sunlight. Storage above 20°C (70°F) should be avoided. The product can settle gradually. Large-scale storage with either gentle agitation or occasional pump recirculation is recommended.

**SAFETY & ENZYME HANDLING**

Inhalation of enzyme dust and mists should be avoided. In case of contact with the skin or eyes, promptly rinse with water for at least 15 minutes. For detailed handling information, please refer to the appropriate Material Safety Data Sheet, the Enzyme Technical Association (ETA) handbook Working Safely With Enzymes, and the Association of Manufacturers and Formulators of Enzyme Products (Amfep) handbook Guide to the Safe Handling of Microbial Enzyme Preparations. All are available from DuPont®.

**TECHNICAL SERVICE**

Information covering specific applications of this product is available. DuPont® will work with customers to enhance processes and solve problems. Let us know what you need, and we will assist you.

**EFFEYCT OF pH AND TEMPERATURE**

ACCELLERASE® 1500 enzyme complex has the best operational stability in the following ranges:

**Temperature:**  50 – 65°C (122 – 149°F)

**pH:**  4.0 – 5.0

ACCELLERASE® 1500 enzyme complex is easily inactivated at temperatures above 70°C (158°F) or at pH levels above 7.0 or below 3.9.
FOR MORE INFORMATION

USA and Canada
Rochester, New York
Telephone: +1 800 847 5311

Europe, Africa and Middle East
Leiden, The Netherlands
Telephone: +31 71 5686 168

Latin America
Buenos Aires, Argentina
Telephone: +54 11 5199 9550

Asia/Pacific
Singapore
Telephone: +65 6511 5600

Shanghai, P.R. China
Telephone: +86 21 2307 9588

Mumbai, India
Telephone: +91 22 2825 8713

Web Address: www.accellerase.dupont.com