Biofiles

Volume 5, Number 5

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Enzymes and Reagents for Alternative Energy

Metabolic strategies in alternative energy

Enzyme-based fuel cells

Lignocellulosic depolymerization

Enzymes for biofuel research

Reagents for ethanol and biodiesel analysis

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Introduction

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Leveraging nature's metabolic strategies for tomorrow's energy resources.

Although most of the world's population understands that carbon dioxide is the predominant end product of energy production from combustible fuels, most of us forget that the "raw material" for most of our combustible fuel sources is also atmospheric carbon dioxide. Photosynthetic carbon dioxide fixation is most often the first step in the synthesis of both our renewable fuels and non-renewable fossil fuels. Even much of our hydrogen production is an indirect product of carbon dioxide fixation as it is often derived from fossil fuels.

The basic differences between non-renewable fossil fuels and our "new generation" renewable fuels is time, energy input and mass. Our non-renewable fuels, such as petroleum and coal required thousands of years of collection of solar energy to power carbon fixation for the biosynthesis of enormous amounts of biomass. That biomass then underwent millions of years of anaerobic decomposition to yield today's fossil fuels. In our attempt to circumvent or speed up this process, we are finding natural "renewable analogs" to some of our fossil fuels, i.e., ethanol and butanol to replace or supplement gasoline and fatty acid esters to replace diesel.

However, we have not advanced far enough in our manufacturing technology to divest ourselves of the need for biological systems to do the complicated work for us. Current processes for ethanol production rely on plants to fix carbon dioxide, synthesize a "common currency" molecule such as glucose, and then needlessly polymerize this glucose into starch or cellulose. Then we often rely on microbial and enzymatic hydrolysis of these biopolymers to give back glucose and then ferment it to yield ethanol. Sugarcane ethanol employs a slightly simpler path via sucrose.

Our challenge for the future may be to find the "straightest metabolic line" between carbon dioxide and ethanol, butanol, fatty acids or even hydrogen. The ideal route to ethanol production might be to devise an enzymatic or cellular system to fix carbon dioxide and take the direct route from ribose-1,5-bis-phosphate to pyruvate to ethanol and bypass glucose biosynthesis, polymerization and depolymerization. We have included the Nicholson/IUBMB Metabolic Pathway chart on the following pages illustrating the specific pathways related to carbon dioxide fixation and glucose, cellulose, pectin, starch, lignin and ethanol production highlighted in yellow.

Enzymes and their metabolic pathways enter the energy equation from other directions. Microbial strains are being discovered that can ferment cellulosic biomass to yield hydrogen. Some photosynthetic microbes, such as algae, are capable of splitting water to produce hydrogen utilizing solar energy. Hydrogenases are being targeted not only for hydrogen production but also for the oxidation of H_2 in fuel cells. Immobilized enzyme-based fuel cell batteries can utilize multi-enzyme systems that mimic *in vivo* glycolytic and Krebs Cycle pathways. Immobilized enzymes are also being studied for the sequestration of carbon dioxide emissions.

Engineering organisms and enzyme systems to maximize biofuel production could require insertion of exogenous enzymatic pathways from various organisms into a new host. In addition, alternative metabolic pathways such as the mevalonate and isoprenoid pathways are being studied as sources of biofuel.

This issue of BioFiles is devoted to how Sigma-Aldrich enzymes and reagents are involved in many of these areas of research. As summarized above and illustrated below, many of these areas require an understanding of metabolic pathways and enzymatic processes.

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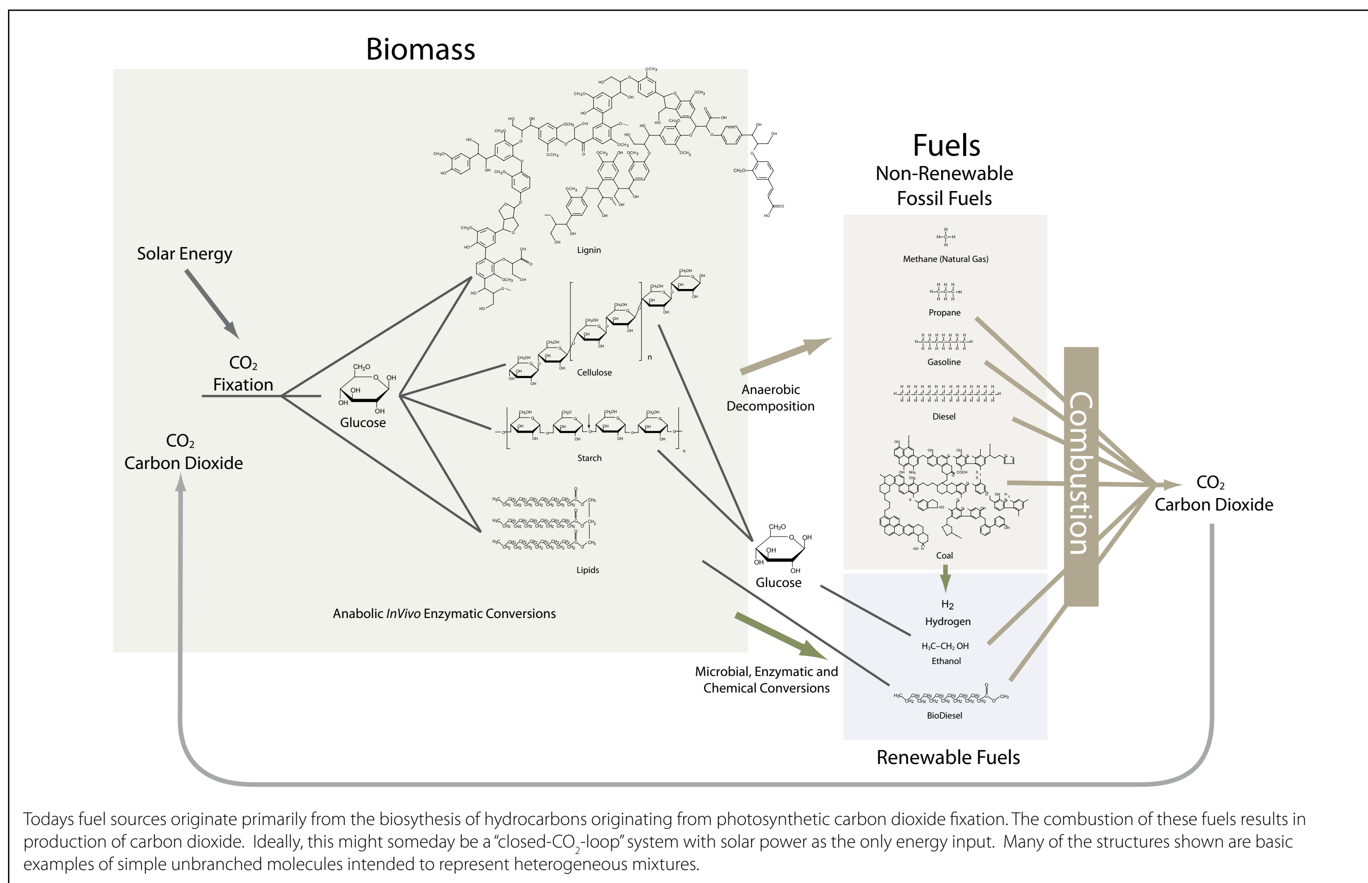

Todays fuel sources originate primarily from the biosythesis of hydrocarbons originating from photosynthetic carbon dioxide fixation. The combustion of these fuels results in production of carbon dioxide. Ideally, this might someday be a "closed-CO_2-loop" system with solar power as the only energy input. Many of the structures shown are basic examples of simple unbranched molecules intended to represent heterogeneous mixtures.

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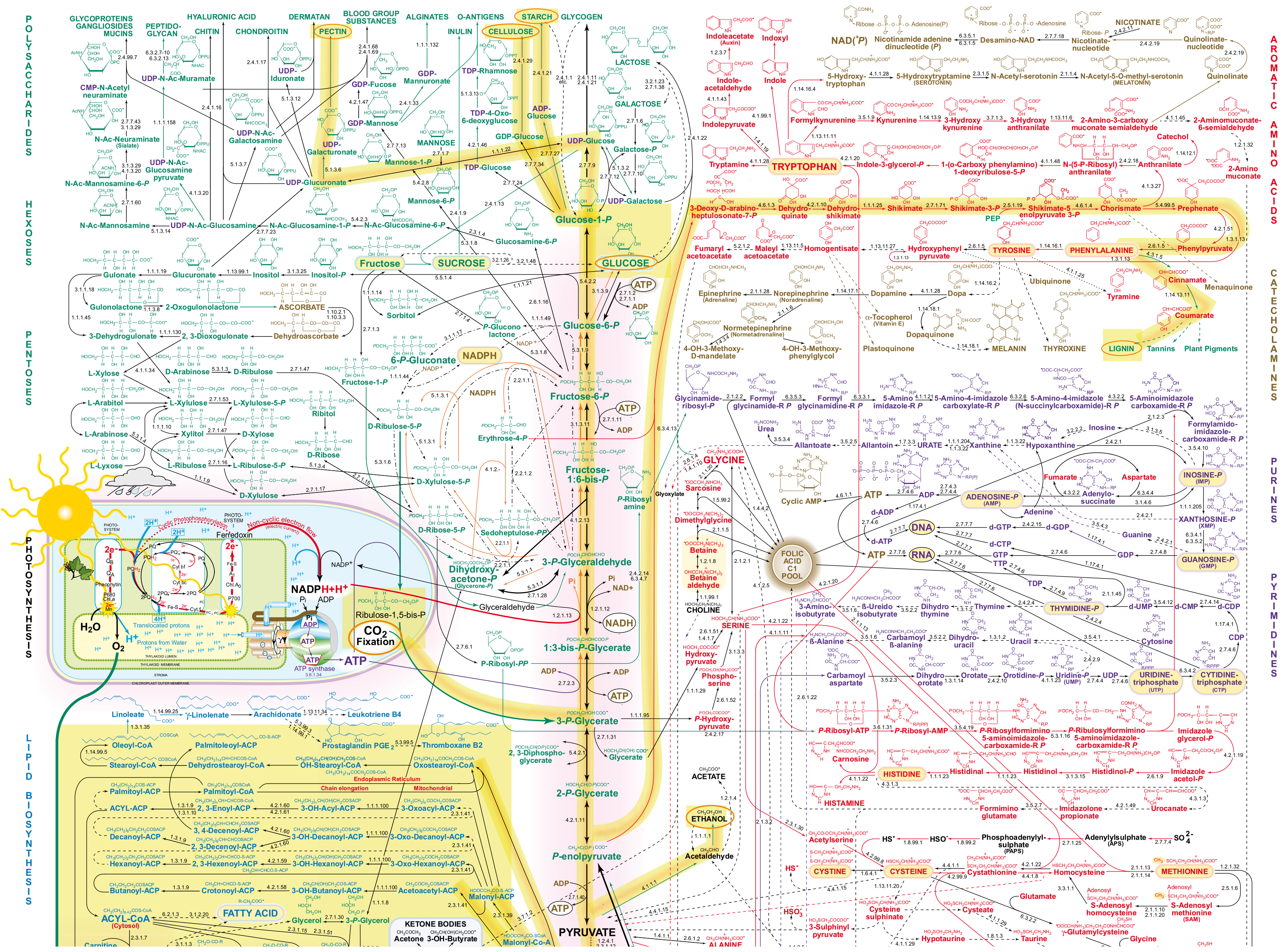
Metabolic Origins of Fuel



BIOSYNTHESIS

LIPID DEGRADATION

PHOSPHOLIPIDS

ISOPRENOIDS

STEROIDS

PORPHYRINS

AMINO ACIDS

LEGEND

Carbohydrates
Amino Acids
Lipids
Purines & Pyrimidines
Vitamins Co-enzymes & Hormones
Pentose Phosphate Pathway
Photosynthesis Dark Reactions

COMPARTMENTATION

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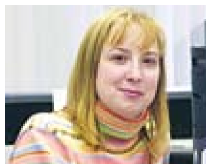


Advancements in Enzyme-Based Fuel Cell Batteries, Sensors and Emissions Reduction Strategies
Dr. Shelley Minteer, Ph. D.

Advancements in Enzyme-Based Fuel Cell Batteries, Sensors and Emissions Reduction Strategies

Sigma® Life Science and Aldrich Materials Science merge to help researchers completely rethink tomorrow's immobilized enzyme technologies.

Today's fuel cells and batteries lack the ability to produce electrical power for the long periods of time we desire and commonly incorporate toxic or precious metals to produce and store their energy.

Enzyme-based fuel cells have potentially higher energy density than traditional batteries using much more environmentally compatible materials. While the idea of enzyme-based biofuel cells is not a new concept, recent developments have captured the interests of the research community. Dr. Shelley Minteer at Saint Louis University Department of Chemistry is one of the pioneers in this area. Dr. Minteer and her team have developed a new enzyme immobilization technology that has begun to revolutionize the way we think about using enzymes for long-term catalytic applications.

Dr. Minteer's enzyme immobilization strategy encapsulates and stabilizes Sigma enzymes in modified Nafion® polymer matrices from Aldrich. The efficacy of Dr. Minteer's immobilized enzymes can be measured in years compared to days for other biofuel cell technologies. Dr. Minteer has successfully demonstrated the use of several metabolic fuels such as glycerol, fatty acids, ethanol and other alcohols, pyruvate and glucose to provide the fuel source, while a wide variety of enzymes and coupled multienzyme systems provide the catalytic power to convert the fuel to electrical current.

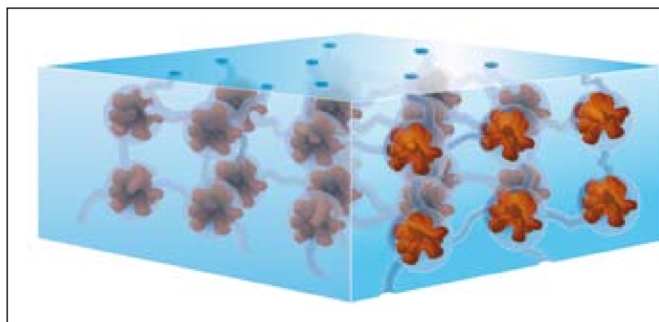
In addition to synthetic polymer supports, Dr. Minteer and her collaborator, Dr. Michael Cooney of the University of Hawaii, have immobilized dehydrogenases on macroporous chitosan scaffolds. The scaffolds were fabricated from solutions of native and hydrophobically modified chitosan polymers through the process of thermally induced phase separation. The hydrophobically modified chitosan is proposed to possess amphiphilic micelles into which the enzyme can be encapsulated and retained.

One of Dr. Minteer's basic biofuel cell batteries employs an ethanol/alcohol dehydrogenase system to generate a proton gradient across the conductive membrane. They have also refined these dehydrogenase systems by replacing traditional NAD(H) cofactor-dependent dehydrogenases with pyrroloquinoline quinone(PQQ)-dependent dehydrogenases to improve efficiency. Other multienzyme coupled systems use classical glycolytic and Krebs cycle enzyme systems and corresponding metabolic substrates to generate power.

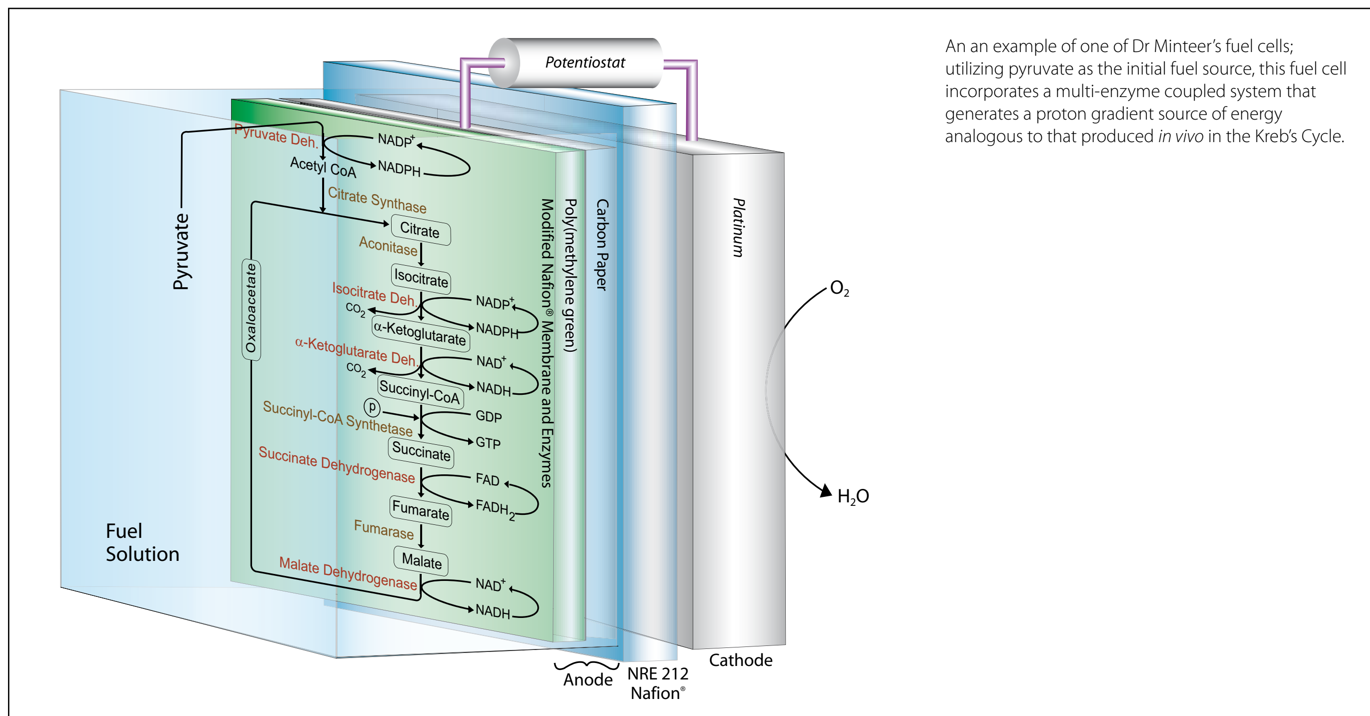
Dr. Minteer's lab has also utilized mitochondria to generate current by metabolism of classic cellular energy sources. The resulting electrons are shuffled through the electron transport chain where they reduce oxygen at cytochrome c oxidase and combine with the protons from oxidation to form water. During this metabolic process it is possible for cytochrome c to undergo direct electron transfer to a carbon electrode and complete oxygen reduction at a platinum cathode.

Taking advantage of the fact that nitroaromatics can decouple the inhibition of pyruvate metabolism, mitochondria-modified electrodes have also been used to electrochemically sense the presence of nitroaromatic explosive compounds. These oligomycin inhibited mitochondria-modified electrodes were employed as the bioanode in a pyruvate/air biofuel cell. This concept could be used as a traditional electrochemical sensor or as a self-powered sensor.

Dr. Minteer's Nafion-based immobilization technology is also addressing the reduction of fluegas emissions from coal-fired power plants using carbonic anhydrase to sequester CO_2.



Enzymes immobilized in Nafion® membrane are encapsulated in the polymer resulting in increased stability while retaining active site availability and optimized orientation.



An an example of one of Dr Minteer's fuel cells; utilizing pyruvate as the initial fuel source, this fuel cell incorporates a multi-enzyme coupled system that generates a proton gradient source of energy analogous to that produced *in vivo* in the Kreb's Cycle.

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Selected Enzymes for Biofuel Cell Research

Enzyme-based biofuel cells are incorporating a wide variety of enzymes. Sigma-Aldrich manufactures hundreds of enzymes, many of which may have potential applications in biofuel cells. For a complete list of enzymes, data sheets, assay protocols, and other enzyme-related technical resources visit the web-based Sigma Enzyme Explorer at sigma-aldrich.com/enzymeexplorer. The Enzyme Explorer also features the IUBMB on-line metabolic pathway chart with over 500 links to enzymes, substrates and cofactors.

The following products are only a small sample of the enzymes available from Sigma-Aldrich of potential use in biofuel cell research.

▼ Alcohol Dehydrogenase

ADH

Alcohol Dehydrogenase from *Parvibaculum lavamentivorans*

- **recombinant, expressed in *Escherichia coli*, activity: ≥40.0 units/mg**

One unit corresponds to the amount of enzyme which reduces 1 µmole ethyl acetate per minute at pH 7.0 and 30 °C.

75449-1ML	1 mL
75449-5ML	5 mL

Alcohol Dehydrogenase from *Saccharomyces cerevisiae*

Alcohol:NAD⁺ oxidoreductase; Alcohol Dehydrogenase from yeast
[9031-72-5]

A 141 kDa tetramer containing 4 equal subunits. The active site of each subunit contains a zinc atom. Each active site also contains 2 reactive sulfhydryl groups and a histidine residue.

Isoelectric point: 5.4–5.8

Optimal pH: 8.6–9.0

Substrates: Yeast ADH is most active with ethanol and its activity decreases as the size of the alcohol increases or decreases. Branched chain alcohols and secondary alcohols also have very low activity.

K_M (ethanol) = 2.1×10^{-3} M

K_M (methanol = 1.3×10^{-1} M

K_M (isopropanol) = 1.4×10^{-1} M

Inhibitors: Compounds that react with free sulfhydryls, including N-alkylmaleimides and iodoacetamide.

Zinc chelator inhibitors, including 1,10-phenanthroline, 8-hydroxyquinoline, 2,2′-dipyridyl, and thiourea.

Substrate analogue inhibitors, including β-NAD analogs, purine and pyrimidine derivatives, chloroethanol, and fluoroethanol.

Extinction Coefficient: $E^{1\%}$ = 14.6 (water, 280 nm)

One unit will convert 1.0 µmole of ethanol to acetaldehyde per min at pH 8.8 at 25 °C.

- **lyophilized powder (contains buffer salts), activity: ≥300 units/mg protein**

Solids containing ≤ 2% citrate buffer salts suitable for conventional determination of β-NAD, β-NADH, ethanol and acetaldehyde

Contains bound β-NAD and β-NADH and is not suitable for the recycling microassay of β-NAD and β-NADH. If you require ADH for this purpose, see **Cat. No. A3263**.

A7011-7.5KU	7500 units
A7011-15KU	15000 units
A7011-30KU	30000 units
A7011-75KU	75000 units
A7011-150KU	150000 units
A7011-300KU	300000 units

- **activity: ≥300 units/mg protein**

Solids containing <2% citrate buffer salts suitable for recycling micro-assay of β-NAD and β-NADH

A3263-7.5KU	7500 units
A3263-15KU	15000 units
A3263-30KU	30000 units
A3263-75KU	75000 units
A3263-150KU	150000 units

Alcohol Dehydrogenase, *Deinococcus radiodurans*, recombinant from *E. coli*

ADH
[9031-72-5]

- **activity: ≥10000 units/mL**

One unit corresponds to the amount of enzyme which reduces 1 µmole ethyl-2-oxo-4-phenylbutyrate per minute at pH 7.0 and 37 °C

16892-1ML	1 mL
16892-5ML	5 mL

Alcohol Dehydrogenase, NADP⁺ dependent from *Thermoanaerobium brockii*

Alcohol:NADP⁺ oxidoreductase; TBADH
[9028-12-0]

An extremely thermostable enzyme with broad substrate specificity for alcohols, ketones and acetaldehyde.

One unit will oxidize 1.0 µmole of 2-propanol to acetone per min at pH 7.8 at 40 °C in the presence of NADP⁺.

- **lyophilized powder, activity: 5–15 units/mg protein**

Contains phosphate buffer salts and dithioerythritol

A8435-100UN	100 units
A8435-250UN	250 units

- **lyophilized powder, activity: 30–90 units/mg protein**

Purified

Contains sodium citrate and dithioerythritol.

A9287-100UN	100 units

Alcohol Dehydrogenase ▲

Bilirubin Oxidase from *Myrothecium verrucaria*

Bilirubin:oxygen oxidoreductase
[80619-01-8]

- **lyophilized powder, activity: 15–65 units/mg protein**

One unit will oxidize 1.0 µmole of bilirubin per min at pH 8.4 at 37 °C.

B0390-25UN	25 units
B0390-100UN	100 units

Cytochrome c Oxidase from bovine heart

Ferrocytochrome-c:oxygen oxidoreductase, Complex IV; EC 1.9.3.1
[9001-16-5]

Cytochrome c oxidase is the principal terminal oxidase of high oxygen affinity in the aerobic metabolism of all animals, plants, yeasts and some bacteria.

Supplied as a solution in 25 mM Tris-HCl buffer, pH 7.8, 5 mM EDTA, and 39 mM sodium dodecyl maltoside.

activity: ≥20 units/mg protein

One unit of cytochrome c oxidase will oxidize 1.0 µmole of ferrocytochrome c per min. at 25 °C at pH 6.0.

C5499-250UG	250 µg

D-Fructose Dehydrogenase from *Gluconobacter industrius*

D-Fructose:(acceptor) 5-oxidoreductase
[37250-85-4]

- **lyophilized powder, activity: 400–1,200 units/mg protein**

Lyophilized powder containing citrate-phosphate buffer salts, TRITON® X-100, and stabilizer

One unit will convert 1.0 µmole D-fructose to 5-ketofructose per min at pH 4.5 at 37 °C.

F5152-250UN	250 units

Glucose Dehydrogenase from calf liver

β-D-Glucose: NAD[P]$^+$ 1-oxidoreductase
[9028-53-9]

- **activity: ~15 units/g solid**

Crude powder

One unit will oxidize 1.0 µmole of β-D-glucose to D-glucono-δ-lactone per min at pH 7.6 at 25 °C.

G5625	Inquire

Glucose Dehydrogenase from *Pseudomonas sp.*

[9028-53-9]

- **powder, activity: ≥200 units/mg**

One unit corresponds to the amount of enzyme which will oxidizes 1 µmole β-D-glucose to D-glucono-δ-lactone per minute at pH 8.0 and 37 °C

19359-10MG-F	10 mg

Glucose Dehydrogenase from *Thermoplasma acidophilum*

β-D-Glucose:NAD[P]$^+$ 1-oxidoreductase
[9028-53-9]

- **recombinant, expressed in *Escherichia coli*, ammonium sulfate suspension, activity: ≥100 units/mg protein (glucose substrate), activity: ≥50 units/mg protein (galactose substrate)**

A thermostable enzyme with resistance to organic solvents. Enzyme also shows substantial activity with D-galactose as substrate.

Suspension in 2.8 M $(NH_4)_2SO_4$, 50 mM phosphate buffer, pH 7.0

One unit will oxidize 1.0 µmole of D-glucose to D-glucono-δ-lactone per min at pH 7.0 at 37 °C in the presence of NADP$^+$.

G5909-100UN	100 units
G5909-500UN	500 units

Glucose Oxidase from *Aspergillus niger*

G.Od.; GOx; β-D-Glucose:oxygen 1-oxidoreductase
[9001-37-0]

Molecular Weight: 160 kDa (gel filtration)

pI: 4.2

Extinction coefficient: $E^{1\%}$ = 16.7 (280 nm)

Glucose oxidase from *Aspergillus niger* is a dimer consisting of 2 equal subunits with a molecular mass of 80 kDa each. Each subunit contains one flavin adenine dinulceotide moiety and one iron. The enzyme is a glycoprotein containing ~16% neutral sugar and 2% amino sugars. The enzyme also contains 3 cysteine residues and 8 potential sites for N-linked glycosylation.

Glucose oxidase is capable of oxidizing D-aldohexoses, monodeoxy-D-glucoses, and methyl-D-glucoses at varying rates.

The pH optimum for glucose oxidase is 5.5, while it has a broad activity range of pH 4–7. Glucose oxidase is specific for β-D-glucose with a K_M of 33–110 mM.

Glucose oxidase does not require any activators, but it is inhibited by Ag^+, Hg^{2+}, Cu^{2+}, phenylmercuric acetate, and *p*-chloromercuribenzoate. It is not inhibited by the nonmetallic SH reagents: N-ethylmaleimide, iodoacetate, and iodoacetamide.

Glucose oxidase can be utilized in the enzymatic determination of D-glucose in solution. As glucose oxidase oxidizes β-D-glucose to D-gluconolactate and hydrogen peroxide, horseradish peroxidase is often used as the coupling enzyme for glucose determination. Although glucose oxidase is specific for β-D-glucose, solutions of D-glucose can be quantified as α-D-glucose will mutorotate to β-D-glucose as the β-D-glucose is consumed by the enzymatic reaction.

One unit will oxidize 1.0 µmole of β-D-glucose to D-gluconolactone and H_2O_2 per min at pH 5.1 at 35 °C, equivalent to an O_2 uptake of 22.4 µl per min. If the reaction mixture is saturated with oxygen, the activity may increase by up to 100%.

- **lyophilized powder, activity: 100,000–250,000 units/g solid (without added oxygen)**

May contain traces of amylase, maltase, glycogenase, invertase, and galactose oxidase.

Protein determined by biuret

G7141-10KU	10000 units
G7141-50KU	50000 units
G7141-250KU	250000 units
G7141-1MU	1000000 units
G7141-2.5MU	2500000 units

▼ Laccase

[80498-15-3]

Laccase from *Rhus vernicifera*

Benzenediol:oxygen oxidoreductase

- **crude acetone powder, activity: ≥50 units/mg solid**

One unit will produce a ΔA_{530} of 0.001 per min at pH 6.5 at 30 °C in a 3 mL reaction volume using syringaldazine as substrate.

L2157-10KU	10000 units

Laccase from *Trametes versicolor*

former nomenclature: *Coriolus versicolor*

- **powder, activity: >20 units/mg**

One unit corresponds to the amount of enzyme which converts 1 µmole of catechol per minute at pH 4.5 and 25 °C

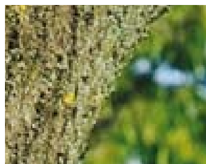
53739-100MG-F	100 mg
53739-1G-F	1 g

- **powder, activity: ≥0.5 units/mg**

One unit corresponds to the amount of enzyme which converts 1 µmole of catechol per minute at pH 6.0 and 25 °C

38429-1G	1 g
38429-10G	10 g

Laccase ▲



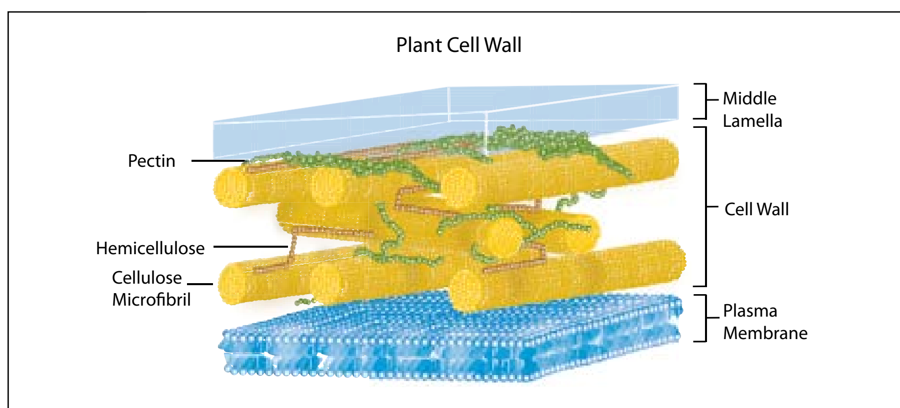
Enzymes and Reagents for Ethanol Research

Enzymes for Lignocellulosic Ethanol Research

Sigma-Aldrich is committed to helping unlock today's challenge of releasing the energy of glucose that nature has cleverly locked into lignocellulosic biomass. To the untrained eye, starch and cellulose structures appear almost identical. Yet the barriers between cellulosic biomass hydrolysis and ethanol production appear have proven to be orders of magnitude more challenging than with starch derived biomass.

Although many of the biochemical aspects of these challenges are well understood, they remain an enigma of nature. The two main, and closely related hindrances to the enzymatic hydrolysis of cellulosic polymers are:

How do we physically dismantle biomass on a molecular level to yield cellulosic polymers available to hydrolysis by cellulase and other glycolytic enzymes? How do we penetrate the barrier posed by the closely integrated network of lignins surrounding cellulose microfibrils?



Visit the Enzyme Explorer Assay Library

Features over 600 detailed procedures for measuring enzyme activity and related metabolites.

The Library is the result of over ten years of in-house work on analytical procedures developed by Sigma-Aldrich® scientists.

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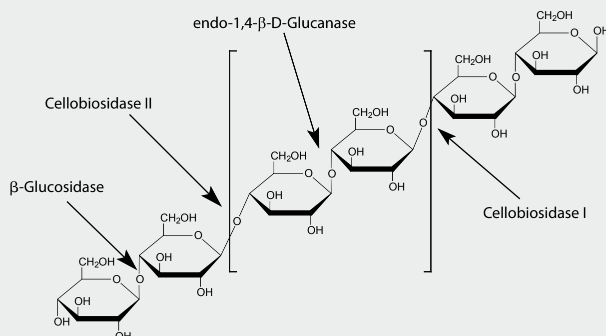
Cellulases and Related Enzyme Activities

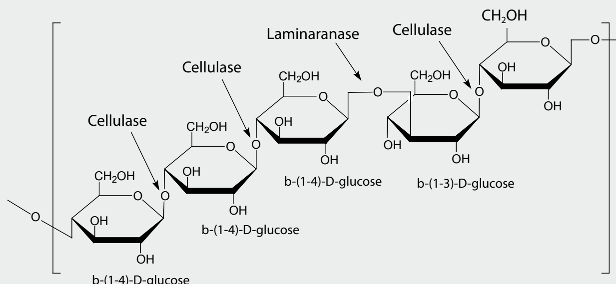


Cellulase catalyzes the endohydrolysis of β–(1→4)-D-glucosidic linkages in cellulose, lichenin and cereal β-D-glucans

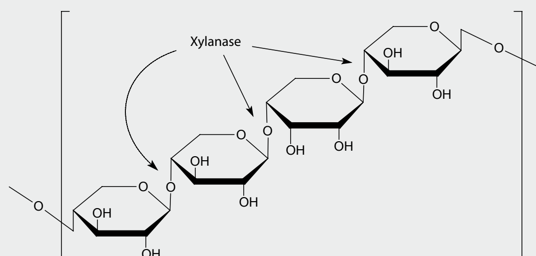
Most cellulase preparations contain various classes of cellulases and related activities.

- The endo-β-(1→4)-D-glucanases (EGs) cleave cellulose in its internal non-terminal regions, yielding oligosaccharides.
- The two forms of cellobiohydrolases (CBH) are considered exo-β-(1→4)-D-glucanases, releasing cellobiose disaccharides from the cellulose chain reducing end (CBHI), and non-reducing end (CBHII).
- β-Glucosidase is an exohydrolase that yields glucose monosaccharides from soluble oligosaccharaides such as cellobiose.

Other activities that can be found in cellulase preparations include xylanase, hemicellulase, and laminarinase.



Cereal β-Glucan is a polymer of β-(1→4)-D-glycopyranosyl units occupying as predominantly cellotriose and cellotetraose separated by single β-(1→3)-D-glycopyranosyl units. Crosslinking can occur within the consecutive cellotriose regions.



Hemicelluloses are a group of plant-derived heteropoly-saccharides associated with cellulose and lignin. The most common hemicelluloses are xylan, glucuronoxylan, arabinoxylan, glucomannan and xyloglucan. In angio-sperms, the principal hemicellulose component, xylan, is a polymer of β–(1→4)-D-xylopyranose. In arabinoxylan, branching occurs at the C2 & C3 postions with a-L-arab-inofunaose. Glucuronoxylan, also found in angiosperms, has the xylan backbone with 4-0 methylglucuronic acid branching. In addition, arabinose branching as well as acetylation may be present. Gymnosperms contain glucomannans comprised primarily of D-mannosyl and D-glucosyl residues..

- Hemicellulase preparations are typically a mixture of glycolytic enzymes containing xylanase, mananase and other activities.
- Xylanase catalyzes the endohydrolysis of β–(1→4)-D-xylosidic linkages in xylans yielding various β–(1→4)-D-xylooligosaccharides.

Cellobiase from *Aspergillus niger*

Cellobiase enzyme preparation obtained by submerged fermentation of an *Aspergillus niger* microorganism. The cellobiase hydrolyzes cellobiose to glucose.

- **Novozyme 188 liquid, activity: ≥250 units/g**

A product of Novozyme Corp.

C6105-50ML	50 mL
C6105-250ML	250 mL

▼ Cellulase

[9012-54-8]

Cellulase from *Aspergillus* sp.

Carezyme 1000L®

activity: ≥1000 units/g

produced by submerged fermentation of a genetically modified *Aspergillus* microorganism

A product of Novozyme Corp.

C2605-50ML	50 mL
C2605-250ML	250 mL

Cellulase from *Trichoderma longibrachiatum*

- **powder, activity: ≥1.0 unit/mg solid**

An acid cellulase with xylanase, pectinase, mannanase, xyloglucanase, laminarase, β-glucosidase, β-xylosidase, α-L-arabinofuranosidase, amylase, and protease activities

One unit will liberate 1.0 µmole of glucose from cellulose in one hour at pH 5.0 at 37 °C (2 hr incubation time).

C9748-100G	100 g

Cellulase from *Trichoderma reesei* ATCC 26921

1,4-(1,3:1,4)-β-D-Glucan 4-glucano-hydrolase

- **lyophilized powder, activity: ≥1 unit/mg solid**

One unit will liberate 1.0 µmole of glucose from cellulose in one hr at pH 5.0 at 37 °C (2 hr incubation time).

C8546-2.5KU	2500 units
C8546-5KU	5000 units
C8546-10KU	10000 units

- **Celluclast® 1.5L aqueous solution, activity: ≥700 units/g**

Produced by submerged fermentation of a selected strain of the fungus *Trichoderma reesei* and catalyzes the breakdown of cellulose into glucose, cellobiose, and higher glucose polymers.

A product of Novozyme Corp.

C2730-50ML	50 mL

Cellulase from *Trichoderma viride*

1,4-(1,3:1,4)-β-D-Glucan 4-glucano-hydrolase

One unit will liberate 1.0 µmole of glucose from cellulose in one hour at pH 5.0 at 37 °C (2 hr incubation time).

- **crude powder, activity: 3–10 units/mg solid**

C9422-5KU	5000 units
C9422-10KU	10000 units

- **Onozuka RS powder, activity: ≥5,000 units/g solid**

Manufactured by Yakult

C0615-1G	1 g

Cellulase ▲

Driselase® from *Basidiomycetes* sp.

[85186-71-6]

Crude powder containing laminarinase, xylanase and cellulase.

- **powder**

D9515-1G	1 g
D9515-5G	5 g
D9515-25G	25 g

▼ β-Glucanase

β-Glucanase from *Aspergillus niger*

[9074-98-0]

- **powder, activity: ~1 units/mg**

One unit corresponds to the amount of enzyme which will release 1 µmole of reducing sugar equivalents (expressed as glucose) per minute at pH 5.0 and 55 °C, using β-D-glucan **(Cat. No. 49102)** as substrate

49101-100MG	100 mg
49101-500MG	500 mg

β-Glucanase from *Bacillus subtilis*

[9074-98-0]

- **powder, activity: ~1 units/mg**

One unit corresponds to the amount of enzyme which will release 1 µmol of reducing sugar equivalents (expressed as glucose) per minute of pH 6.0 and 55 °C, using β-D-glucan (**Cat. No. 49102**) as substrate

49106-100MG	100 mg

β-Glucanase from *Trichoderma longibrachiatum*

A mixture composed mainly of β-1→3 / 1→4-glucanase, xylanase, and cellulase activities. β-glucosidase, β-xylosidase, α-L-arabinofuranosidase,

amylase, and protease activities are also present.

activity: ≥1.0 units/mg solid

One unit will liberate 1.0 µmole of glucose from cellulose in one hr at pH 5.0 at 37 °C (2 hr incubation time).

G4423-100G	100 g

β-Glucanase ▲

β-(1→3)-D-Glucanase from *Helix pomatia*

[9044-93-3]

- **powder, activity: 0.5–1.5 units/mg**

One unit corresponds to the amount of enzyme which liberates 1 µmole of glucose from laminarin (**Cat. No. 61340**) per minute at pH 5.0 and 37 °C

Improved filterability of wines by enzymic decomposition of carbohydrate-containing colloids[1]; Induction of hydrolases as a defense reaction against pathogens, review[2]

Lit cited: 1. Wucherpfennig, K., and Dietrich, H. , *Weinwirtschaft* **118**, 598 (1982); 2. Boller, T. , *UCLA Symp. Mol. Cell Biol., New Ser.* **22**, 247 (1985)

49103-10MG	10 mg
49103-50MG	50 mg

β-Glucosidase from almonds

β-D-Glucoside glucohydrolase
[9001-22-3]

- **lyophilized powder, activity: ≥2 units/mg solid**

Crude

One unit will liberate 1.0 µmole of glucose from salicin per min at pH 5.0 at 37 °C.

G0395-2.5KU	2500 units
G0395-5KU	5000 units
G0395-50KU	50000 units

- **lyophilized powder, activity: 20–40 units/mg solid**

Chromatographically purified

One unit will liberate 1.0 µmole of glucose from salicin per min at pH 5.0 at 37 °C.

G4511-100UN	100 units
G4511-250UN	250 units
G4511-1KU	1000 units

Hemicellulase from *Aspergillus niger*

[9025-56-3]

- **powder, activity: 0.3–3.0 unit/mg solid (using a β-galactose dehydrogenase system and locust bean gum as substrate)**

An undefined mixture of glycolytic enzymes usually containing xylanase, mannanase and other activities.

One unit will produce a relative fluidity change of 1 per 5 minutes using locust bean gum as substrate at pH 4.5 at 40 °C

H2125-150KU	150000 units

▼ Laminarinase

Endo-1,3(4)-β-glucanase
[62213-14-3]

Laminarinase from *Penicillium* sp.

1,3-(1,3:1,4)-β-D-Glucan 3(4)-glucanohydrolase

- lyophilized powder, activity: 5–10 units/mg protein

Lyophilized powder containing acetate buffer salts

One unit will liberate 1.0 mg of reducing sugar (measured as glucose) from laminarin per min at pH 5.0 at 37 °C.

L9259-25UN	25 units

Laminarinase from *Trichoderma* sp.

1,3-[1,3;1,4]-β-D-Glucan 3(4)-glucanohydrolase

View more information on enzymes for complex carbohydrate analysis at *sigma-aldrich.com/enzymeexplorer*

- powder, activity: 100–400 units/g solid

Contains chitinase activity.

One unit will liberate 1.0 mg of reducing sugar (measured as glucose) from laminarin per min at pH 5.0 at 37 °C.

L5272-5UN	5 units
L5272-25UN	25 units

Laminarinase ▲

▼ Xylanase

View more information on enzymes for complex carbohydrate analysis at *sigma-aldrich.com/enzymeexplorer*

Xylanase from *Thermomyces lanuginosus*

[37278-89-0]

Purified endo-β–(1→4)-xylanase from *Thermomyces lanuginosus* produced by submerged fermentation of a genetically modified *Aspergillus oryzae* microorganism

- Pentopan Mono BG® powder, activity: ≥2500 units/g, recombinant, expressed in *Aspergillus oryzae*

A product of Novozyme Corp.

X2753-10G	10 g
X2753-50G	50 g

Xylanase from *Trichoderma viride*

endo-1,4-β-Xylanase; 1,4-β-D-Xylanxylanohydrolase
[9025-57-4]

- lyophilized powder, activity: 100–300 units/mg protein

Contains sorbitol and sodium acetate buffer salts

One unit will liberate 1 µmole of reducing sugar measured as xylose equivalents from xylan (**Cat. No. X0627**) per min at pH 4.5 at 30 °C.

X3876-250UN	250 units
X3876-1KU	1000 units

Xylanase ▲

endo-1,4-β-Xylanase from *Trichoderma longibrachiatum*

1,4-β-D-Xylanxylanohydrolase

Primary activity is an acid-neutral endo-1,4-β-D-xylanase, additional activities include β-glucanase, cellulase, pectinase, mannanase, xyloglucanase, laminarase, β-glucosidase, β-xylosidase, α-L-arabinofuranosidase, amylase, and protease.

activity: ≥1.0 units/mg solid

One unit will liberate 1 µmole of reducing sugar measured as xylose equivalents from xylan (**Cat. No. X0627**) per min at pH 4.5 at 30 °C.

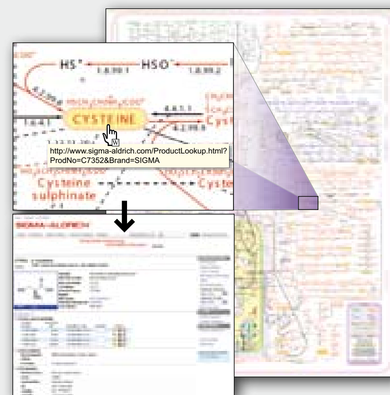
X2629-100G	100 g

Online Metabolomics Resource

Use the IUBMB–Sigma-Aldrich Interactive Metabolic Pathway Chart to find the Metabolite Standards you need.

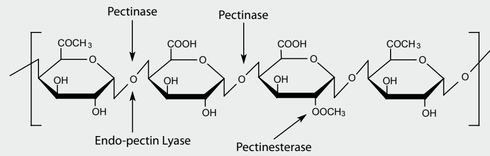
The Metabolic Pathways Map contains over 500 hyperlinks to Sigma product listings. Just click on the metabolite name or the enzyme's E.C. number to access product information.

You can access the chart at
sigma-aldrich.com/metpath

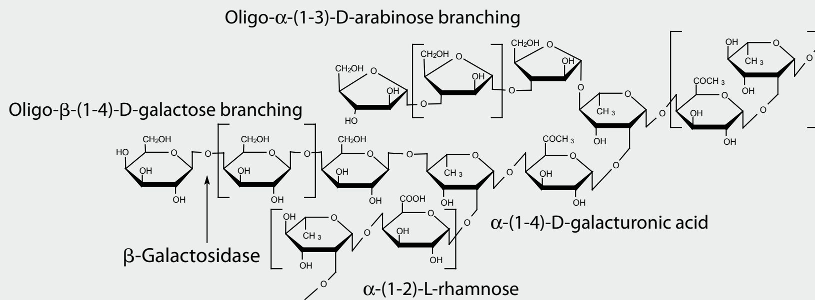


Enzymes for Pectin Hydroylsis

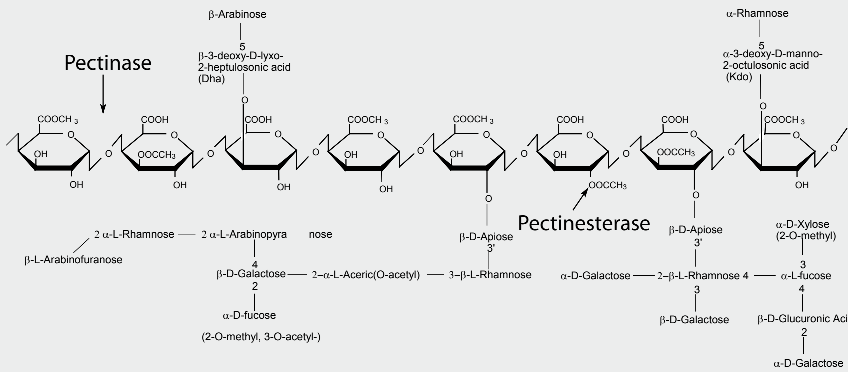
Pectins are complex-branched heteropolysaccharides primarily containing an α-(1→4) polygalacturonic acid backbone which can be randomly acetylated and methylated. Three different pectins have been isolated from plant cell walls.



Homogalacturonan is a composed of a poly-α–(1→4)-galacturonic acid backbone with random partial methylation and acetylation.



Rhamnogalacturonan I is composed of alternating α–(1→2)-L-rhamnosyl-α–(1→4)-D-galacturonosyl backbone with two types of branching composed of a ribofuranose or galactose oligomers.



Rhamnogalacturonan II is composed of poly-α–(1→4)-D-galacturonic acid backbone with random partial methylation, acetylation and four types of branching.

▼ Pectinase

Pectinase from *Aspergillus aculeatus*

▶ Pectinex® Ultra SPL aqueous solution, activity: ≥9,500 units/mL

Highly active pectolytic enzyme preparation produced by a selected strain of *Aspergillus aculeatus*

A product of Novozyme Corp.

P2611-50ML	50 mL
P2611-250ML	250 mL

Pectinase from *Aspergillus niger*

Polygalacturonase solution from *Aspergillus niger*; Poly-(1,4-α-D-galacturonide) glycanohydrolase
[9032-75-1]

Used in plant protoplast preparation to digest cell wall prior to organelle isolation.

▶ aqueous glycerol solution, activity: ≥5 units/mg protein (Lowry)

Solution in 40% glycerol

One unit will liberate 1.0 μmole of galacturonic acid from polygalacturonic acid per min at pH 4.0 at 25 °C.

P4716-5KU	5000 units
P4716-10KU	10000 units
P4716-25KU	25000 units
P4716-100KU	100000 units

Pectinase from *Aspergillus niger*

▶ Pectinex 3XL® aqueous solution

Pectolytic enzyme preparation produced from a selected strain of *Aspergillus niger*: contains mainly pectintranseliminase, polygalacturonase, and pectinesterase and small amounts of hemicellulases and cellulases.

A product of Novozyme Corp.

P2736-50ML	50 mL
P2736-250ML	250 mL

Pectinase from *Rhizopus* sp.

Poly-(1,4-α-d-galacturonide) glycanohydrolase; Poly-galacturonase
[9032-75-1]

Used in plant protoplast preparation to digest cell wall prior to organelle isolation.

▶ Macerozyme R-10 powder, activity: 400–800 units/g solid

A source of pectinase activity, also containing cellulase and hemicellulase activities.

One unit will liberate 1.0 μmole of galacturonic acid from polygalacturonic acid per min at pH 4.0 at 25 °C.

P2401-500UN	500 units
P2401-1KU	1000 units
P2401-5KU	5000 units

Pectinase ▲

Pectolyase from *Aspergillus japonicus*

Reported to contain two types of pectinase, endopolygalacturonase (EC3.2.1.15), endopectin lyase (EC4.2.2.10) and a maceration stimulating factor.

Used in plant protoplast preparation to digest cell wall prior to organelle isolation.

▶ lyophilized powder, activity: ≥0.3 units/mg solid

One unit will liberate 1.0 μmole of galacturonic acid from polygalacturonic acid per min at pH 5.5 at 25 °C.

P3026-100MG	100 mg
P3026-250MG	250 mg
P3026-1G	1 g

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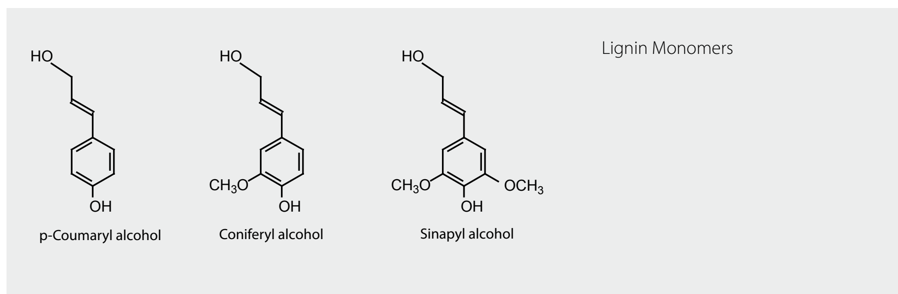
Enzymes for Lignin Depolymerization

To some, lignin appears to be a foreign substance resembling a randomly modified polystyrene polymer, not resembling any of the more familiar biopolymers composed of carbohydrates, amino acids or nucleotides. However, next to cellulose, it is the second most abundant biopolymer on earth comprising as much as 30% of the dry mass of wood. Unlike other biopolymers, enzymatic degradation is oxidative versus hydrolytic and is limited to a select group of fungi and bacteria. Lignins appear to be heterogeneous and random with regards to degree of polymerization, branching, and monomer composition and sequence.



Lignin

Laccase

[80498-15-3]

Laccase from *Rhus vernicifera*

Benzenediol:oxygen oxidoreductase

- crude acetone powder, activity: ≥50 units/mg solid

One unit will produce a ΔA_{530} of 0.001 per min at pH 6.5 at 30 °C in a 3 mL reaction volume using syringaldazine as substrate.

L2157-10KU	10000 units

Laccase from *Trametes versicolor*

former nomenclature: *Coriolus versicolor*

- powder, activity: >20 units/mg

One unit corresponds to the amount of enzyme which converts 1 µmole of catechol per minute at pH 4.5 and 25 °C

53739-100MG-F	100 mg
53739-1G-F	1 g

- powder, activity: ≥0.5 units/mg

One unit corresponds to the amount of enzyme which converts 1 µmole of catechol per minute at pH 6.0 and 25 °C

38429-1G	1 g
38429-10G	10 g

Laccase, *Coriolus versicolor*, CLEA

- Laccase, *Coriolus versicolor*, cross-linked enzyme aggregate activity: ≥0.3 units/mg

One unit corresponds to the amount of enzyme which oxidizes 1 µmol ABTS per minute at pH 4.5 and 25°C

38837-100MG	100 mg

Laccase

Lignin Peroxidase

LiP; Peroxidase, lignin; Ligninase
[42613-30-9]

- powder, activity: >0.1 units/mg

One unit corresponds to the amount of enzyme, which oxidizes 1 µmole 3.4-dimethoxybenzyl alcohol per minute at pH 3.0 and 30 °C

42603-10MG-F	10 mg

Manganese Peroxidase from *Nematoloma frowardii*

[114995-15-2]

41563	Inquire

Manganese peroxidase from white-rot fungus (*Phanerochaete chrysosporium*)

MnP; Peroxidase, manganese; Manganese-dependent lignin peroxidase
[114995-15-2]

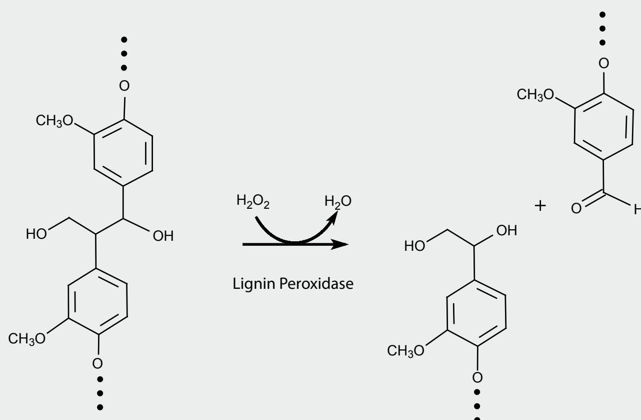
- powder, activity: ≥20 U/g

only partially soluble in water or buffer

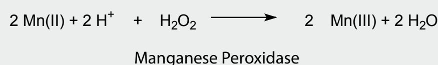
One unit corresponds to the amount of enzyme, which oxidizes 1 µmole Mn^{2+} per minute to Mn^{3+} at pH 4.5 and 25 °C

This product is supplied as a mixture of isozymes, mol. mass (40–65 kDa).

93014-10MG-F	10 mg



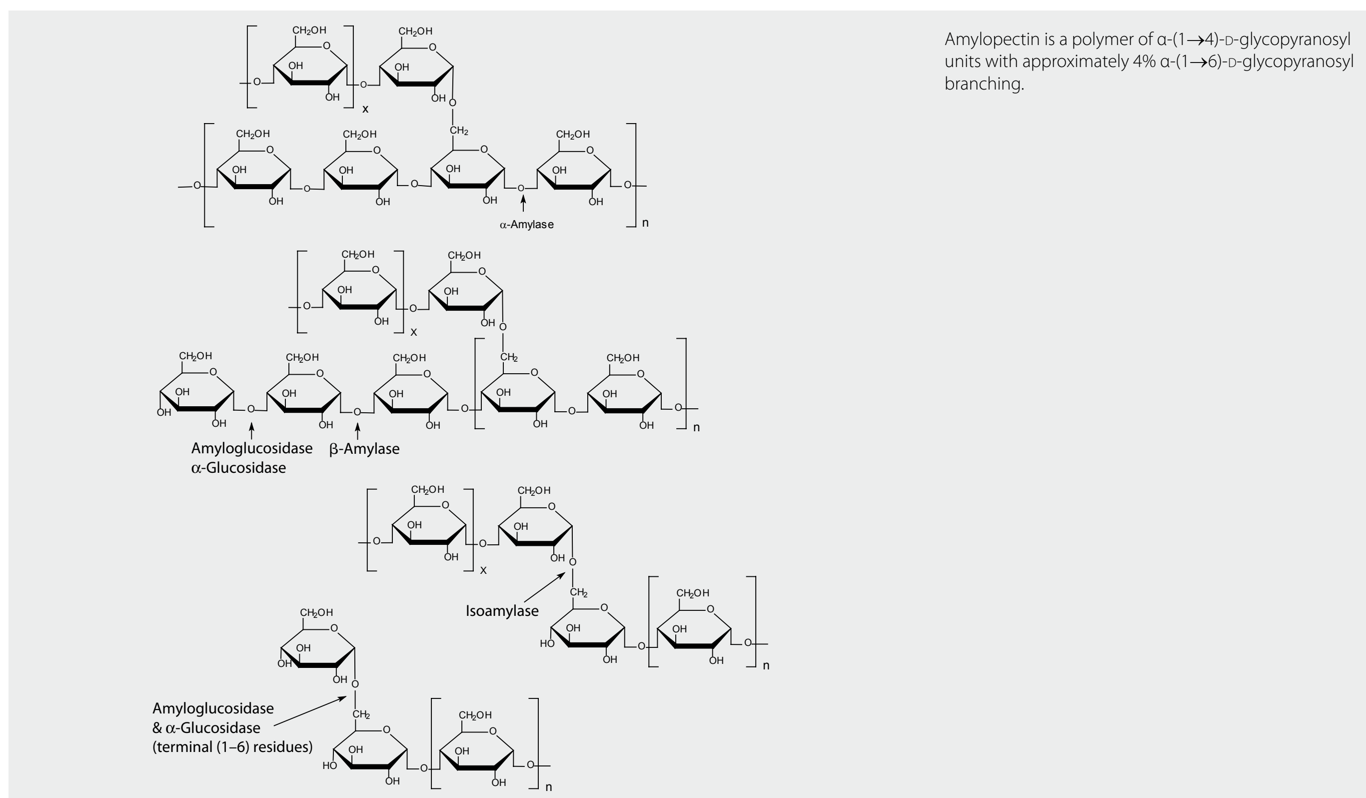
Lignin peroxidase catalyzes the oxidative cleavage of carbon-carbon and ether bonds in lignin-related compounds.



Manganese Peroxidase

Manganese peroxidase is a hemoprotein involved in the oxidative degradation of lignin in white rot basidiomycetes.

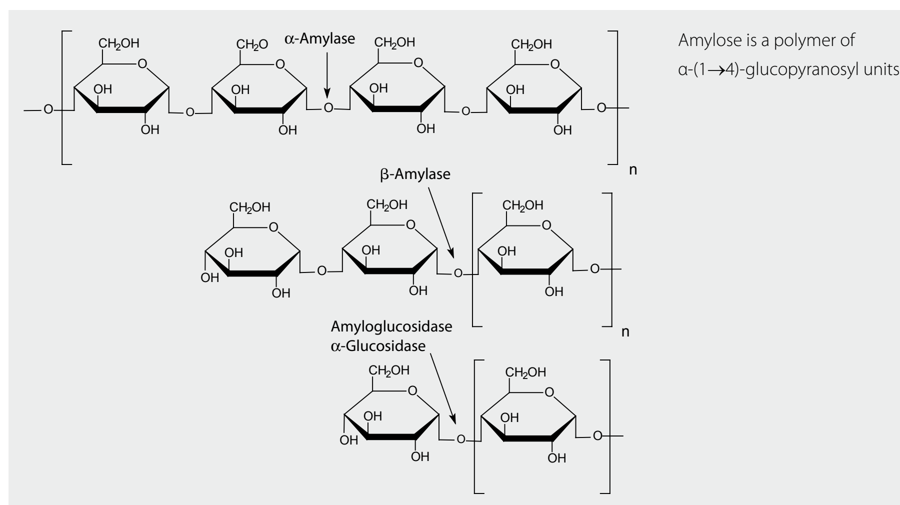
Enzymes for Starch Hydrolysis



Amylopectin is a polymer of α-(1→4)-D-glycopyranosyl units with approximately 4% α-(1→6)-D-glycopyranosyl branching.

Enzymatic hydrolysis of starch-based biomass to yield monomeric glucose is often a multi-enzyme process and can involve α-amylase, β-amylase, amyloglucosidase, isoamylase and α-glucosidase.

- α-Amylase catalyzes the endohydrolysis of α–(1→4)-D-glucosidic linkages in polysaccharides containing three or more α–(1→4)-linked D-glucose units.
- β-Amylase catalyzes the exohydrolysis of α–(1→4)-D-glucosidic linkages in polysaccharides resulting in the successive liberation of maltose units from the non-reducing ends of the chains.
- Amyloglucosidase (glucoamylase) catalyzes the hydrolysis of terminal α–(1→4)-D-glucose residues successively from the non-reducing ends of maltooligo- and polysaccharides with release of β-D-glucose. Most forms of the enzyme can rapidly hydrolyze α–(1→6)-D-glucosidic bonds when the next bond in the sequence is 1→4- and some preparations of this enzyme hydrolyze 1→6- and 1→3-α-D-glucosidic bonds in other polysaccharides.
- Isoamylase catalyzes the hydrolysis of α–(1→6)-D-glucosidic branch linkages in amylopectin and β-limit dextrins.
- α-Glucosidase catalyzes the hydrolysis of terminal 1→4-linked α-D-glucose residues successively from the non-reducing ends of maltooligo- and to a lesser extent polysaccharides with release of β-D-glucose. Most forms of the enzyme can slowly hydrolyze α–(1→6)-D-glucosidic bonds.



Amylose is a polymer of α-(1→4)-glucopyranosyl units.

α-Amylase

α-Amylase from *Aspergillus oryzae*

1,4-α-D-Glucan-glucanohydrolase
[9001-19-8]

- **lyophilized powder, activity: 150–250 units/mg protein (biuret)**

Crude

One unit will liberate 1.0 mg of maltose from starch in 3 min at pH 6.9 at 20 °C.

A6211	Inquire

- **Fungamyl® 800 L aqueous solution, activity: ≥800 FAU/g**

A product of Novozyme Corp.

A8220-50ML	50 mL
A8220-250ML	250 mL

- **Taka-Diastase from *Aspergillus oryzae*; Taka-Amylase A powder, activity: ~1.5 units/mg (~0.2 U acc. to Willstätter)**

One unit corresponds to the amount of enzyme which liberates 1 µmol maltose per minute at pH 6.0 and 25 °C (starch acc. to Zulkowsky, **Fluka No. 85642**, as substrate).

Molecular weight[1]; Product distribution in the hydrolysis of amylose[2]; Application in (selective) hydrolysis/condensation of glycosidic bonds[3]; Suitable for use in thiamine determination[4].

Lit cited: 1. T. Takagi, *J. Biochem.* **89**, 363 (1981); 2. H. Kondo et al., *J. Biochem.* **87**, 1053 (1980); 3. K. Fujita et al., *Bull. Chem. Soc. Jpn.* **62**, 3150 (1989); 4. P.W. Defibaugh, *J. Assoc. Off. Anal. Chem.* **70**, 514 (1987)

86250-100G	100 g
86250-500G	500 g

- **spray-dried (powder), activity: ≥150 units/mg protein (biuret)**

One unit will liberate 1.0 mg of maltose from starch in 3 min at pH 6.9 at 20 °C.

A9857-250KU	250000 units
A9857-1MU	1000000 units
A9857-5MU	5000000 units

α-Amylase from *Bacillus* sp.

- **ammonium sulfate suspension, activity: 40–90 units/mL packed gel**

Suspension in 2.0 M $(NH_4)_2SO_4$, pH 7.0

One unit will liberate 1.0 µmole of maltose from starch per min at pH 6.9 at 30 °C. (Each unit is equivalent to 0.7 of mg maltose liberated per 3 min. at 20 °C)

A0909	Inquire

α-Amylase from *Bacillus amyloliquefaciens*

1,4-α-D-Glucan Glucano-hydrolase
[9000-85-5]

An endoamylase that randomly hydrolyzes α-(1→4)-glycosidic linkages in amylose and amylopectin. The breakdown products are oligosaccharides and dextrins of varying chain length. This enzyme is active at high temperatures (70–90 °C).

One unit is the amount of enzyme which dextrinizes 5.26 g of dry starch per hour under standard conditions.

- **BAN™ 240L liquid, activity: ≥250 units/g**

A product of Novozyme Corp.

A7595-50ML	50 mL
A7595-250ML	250 mL

α-Amylase from *Bacillus licheniformis*

1,4-α-D-Glucan-glucanohydrolase
[9000-85-5]

One unit will liberate 1.0 mg of maltose from starch in 3 min at pH 6.9 at 20 °C.

- **Termamyl® 120 saline solution, activity: 500–1,000 units/mg protein (biuret)**

Aqueous solution containing approx. 15% sodium chloride and 25% sucrose.

Reported to be heat stable at temperatures as high as ~90 °C.

A product of Novozyme Corp.

A3403-500KU	500000 units
A3403-1MU	1000000 units
A3403-5MU	5000000 units

- **lyophilized powder, activity: 500–1,500 units/mg protein**

Heat-stable formulation

Lyophilized powder containing potassium phosphate

A4551-100MG	100 mg
A4551-1G	1 g

α-Amylase from *Bacillus subtilis*

[9000-90-2]

- **powder, activity: ~380 units/mg**

One unit is the amount of enzyme which liberates 1 µmole of maltose per minute at pH 6.9 and 25 °C (using **Cat. No. 85642** as substrate)

Heat stability of bacterial α-amylases[1]; Action pattern on sweet potato starch, amylose and amylopectin[2]; Action on native wheat starch.[3]

Lit cited: 1. J.E. Anderson et al., *J. Food Sci.* **48**, 1622 (1983); 2. P.L. Chang Rupp, S.J. Schwartz, *J. Food Biochem.* **12**, 191 (1988); 3. P. Colonna et al., *Biotechnol. Bioeng.* **31**, 895 (1988).

10069-250MG	250 mg
10069-1G	1 g

- **powder, activity: ~50 units/mg**

One unit corresponds to the amount of enzyme which liberates 1 µmole maltose per minute at pH 6.9 at 25 °C (starch acc. to Zulkowsky, **Cat. No. 85642**, as substrate).

Applications in (selective) hydrolysis/condensations of glycosidic bonds.[1,2]

Lit cited: 1. Y. Takasaki, *Agric. Biol. Chem.* **49**, 1091 (1985); 2. C.S. Su, C.P. Yang, *J. Chem. Technol. Biotechnol.* **48**, 313 (1990)

10070-10G	10 g
10070-50G	50 g

α-Amylase from barley malt

1,4-α-D-Glucan-glucanohydrolase
[9000-90-2]

- **powder**

β-amylase activity: ≥1 unit/mg solid

α-amylase activity: ≥1 unit/mg solid

Package size based on α-amylase activity

One unit will liberate 1.0 mg of maltose from starch in 3 min at pH 6.9 at 20 °C.

A2771	Inquire

α-Amylase from human pancreas

[9000-90-2]

- **lyophilized powder, activity: ≥100 units/mg protein**

Lyophilized from Tris buffer containing NaCl and $CaCl_2$.

Prepared by modified method of Levitzki et al.

One unit will liberate 1.0 mg of maltose from starch in 3 min at pH 6.9 at 20 °C.

A9972-100UG	100 µg

α-Amylase from human saliva

1,4-α-D-Glucan-glucanohydrolase
[9000-90-2]

- **lyophilized powder, activity: 300–1,500 units/mg protein**

Lyophilized powder containing $(NH_4)_2SO_4$ and sodium citrate.

One unit will liberate 1.0 mg of maltose from starch in 3 min at pH 6.9 at 20 °C.

A1031-1KU	1000 units
A1031-5KU	5000 units

- **lyophilized powder, activity: 1,000–3,000 units/mg protein**

Lyophilized powder containing $(NH_4)_2SO_4$ and sodium citrate

Chromatographically purified

One unit will liberate 1.0 mg of maltose from starch in 3 min at pH 6.9 at 20 °C.

A0521-100UN	100 units
A0521-500UN	500 units
A0521-2.5KU	2500 units

α-Amylase from porcine pancreas

Molecular mass: 51–54 kDa.

α-Amylase isolated from porcine pancreas is a glycoprotein. It is a single polypeptide chain of ~475 residues containing two SH groups and four disulfide bridges and a tightly bound Ca^{2+} necessary for stability. Chloride ions are necessary for activity and stability. The pH range for activity is 5.5 to 8.0, with the pH optimum at 7.

One unit will liberate 1.0 mg of maltose from starch in 3 min at pH 6.9 at 20 °C.

- **activity: ≥10 units/mg solid**

Contains lactose

Package size based on α-amylase activity

A3176-500KU	500000 units
A3176-1MU	1000000 units
A3176-2.5MU	2500000 units
A3176-5MU	5000000 units
A3176-10MU	10000000 units

- **DFP Treated, saline suspension, activity: 700–1400 units/mg protein (E $^{1\%}/_{280}$)**

Suspension in 2.9 M NaCl solution containing 3 mM $CaCl_2$

DFP treated. 2× crystallized

View more information on enzymes for complex carbohydrate analysis at *sigma-aldrich.com/enzymeexplorer*

A6255-10MG	10 mg
A6255-25MG	25 mg
A6255-100MG	100 mg

- **DFP treated, ammonium sulfate suspension, activity: ≥500 units/mg protein (E $^{1\%}/_{280}$)**

Suspension in 3.2 M $(NH_4)_2SO_4$, pH 6.1

A2643	Inquire

- **PMSF treated, saline suspension, activity: 700–1400 units/mg protein (E $^{1\%}/_{280}$)**

Suspension in 2.9 M NaCl solution containing 3 mM $CaCl_2$.

2× crystallized

A4268-25MG	25 mg
A4268-100MG	100 mg

α-Amylase ▲

▼ β-Amylase

1,4-α-D-Glucan maltohydrolase
[9000-91-3]

β-Amylase from barley

- **activity: 20–80 units/mg protein (biuret)**

Crude

One unit will liberate 1.0 mg of maltose from starch in 3 min at pH 4.8 at 20 °C.

A7130-10KU	10000 units
A7130-50KU	50000 units
A7130-250KU	250000 units

β-Amylase from sweet potato

- **ammonium sulfate suspension, activity: ≥750 units/mg protein (E $^{1\%}/_{280}$)**

Crystalline suspension in 2.3 M $(NH_4)_2SO_4$

One unit will liberate 1.0 mg of maltose from starch in 3 min at pH 4.8 at 20 °C.

View more information on enzymes for complex carbohydrate analysis at *sigma-aldrich.com/enzymeexplorer*

A7005-10KU	10000 units
A7005-25KU	25000 units
A7005-50KU	50000 units
A7005-100KU	100000 units

β-Amylase ▲

α-Amylase, heat-stable

α-Amylase from *Bacillus licheniformis*; 1,4-α-D-Glucan-glucanohydrolase
[9000-85-5]

- **solution, for use in Total Dietary Fiber Assay, TDF-100A**

A3306-10ML	10 mL

Amylase, Maltogenic from *Bacillus* sp.

Maltogenic Amylase; Glucan 1,4-α-maltohydrolase
Novamyl 1000BG

A2986-10G	10 g

▼ Amyloglucosidase

Exo-1,4-α-glucosidase; 1,4-α-D-Glucan glucohydrolase; Glucoamylase
[9032-08-0]

Amyloglucosidase from *Aspergillus niger*

- **lyophilized, powder, activity: ~70 units/mg**

For glycogen determination in whole yeast cells; Synthesis of hetero-oligosaccharides by glucoamylase in reverse.

One unit corresponds to the amount of enzyme which liberates 1 μmole of glucose per minute at pH 4.8 and 60 °C (starch acc. to Zulkowsky, **Cat. No. 85642**, as substrate).

10115-1G-F	1 g
10115-5G-F	5 g

- **lyophilized powder, activity: 30–60 units/mg protein (biuret)**

Lyophilized powder containing less than 0.02% glucose

One unit will liberate 1.0 mg of glucose from starch in 3 min at pH 4.5 at 55 °C.

A7420-5MG	5 mg
A7420-25MG	25 mg
A7420-100MG	100 mg

- **powder, activity: ~120 units/mg**

One unit corresponds to the amount of enzyme which liberates 1 μmol glucose per minute at pH 4.8 and 60 °C (starch, Fluka No. 85642, as substrate).

Synthesis of hetero-oligosaccharides by glucoamylase in reverse[1].

Lit cited: 1. R.A. Rastall et al., *Biotechnol. Lett.* **13**, 501 (1991)

10113-1G	1 g
10113-5G	5 g

Amyloglucosidase from *Rhizopus* sp.

- **activity: ≥5,000 units/g solid**

One unit will liberate 1.0 mg of glucose from starch in 3 min at pH 4.5 at 55 °C.

Using soluble starch, almost theoretical yields of glucose are obtained.

A7255	Inquire

Amyloglucosidase ▲

▼ α-Glucosidase

α-D-Glucoside glucohydrolase
[9001-42-7]

Protein determined by biuret.

α-Glucosidase from *Bacillus stearothermophilus*

Maltase

- **Maltase lyophilized powder, activity: ≥50 units/mg protein**

Lyophilized powder containing potassium phosphate buffer salt

One unit will liberate 1.0 μmole of D-glucose from *p*-nitrophenyl α-D-glucoside per min at pH 6.8 at 37 °C.

Activity using maltose as substrate at pH 6.0 at 25 deg C is ~2X greater than that obtained using p-nitrophenyl-α-D-glucoside as substrate at pH 6.8 at 37 °C.

G3651-250UN	250 units

α-Glucosidase from rice

Maltase

- **ammonium sulfate suspension, activity: 40–80 units/mg protein**

Suspension in 2.8 M $(NH_4)_2SO_4$ solution

One unit will convert 1.0 μmole of maltose to 2.0 μmoles of D-glucose per min at pH 4.0 at 37 °C.

G9259-100UN	100 units

α-Glucosidase from *Saccharomyces cerevisiae*

α-D-Glucosidase; Maltase from yeast Hydrolysis of terminal, non-reducing 1→4-linked D-glucose residues with release of D-glucose.

For the determination of α-amylase and the synthesis of various 1′-*O*-sucrose and 1-*O*-fructose esters

- **recombinant, expressed in unspecified host, lyophilized powder, activity: ≥125 units/mg protein**

Lyophilized powder containing potassium phosphate buffer salt pH 7.15 and approx. 70% lactose

One unit will liberate 1.0 µmole of D-glucose from *p*-nitrophenyl α-D-glucoside per min at pH 6.8 at 37 °C.

G0660-750UN	750 units

- **lyophilized powder, activity: ≥10 units/mg protein (using *p*-nitrophenyl α-D-glucoside as substrate.)**

Sold on basis of *p*-nitrophenyl α-D-glucoside units.

One unit will liberate 1.0 µmole of D-glucose from p-nitrophenyl α-D-glucoside per min at pH 6.8 at 37 °C.

View more information on enzymes for complex carbohydrate analysis at *sigma-aldrich.com/enzymeexplorer*

G5003-100UN	100 units
G5003-1KU	1000 units

α-Glucosidase ▲

Ethanol Analysis Reagents

ASTM™ D5501 Denatured Fuel Ethanol Standards Kit

Use this quantitative calibration standard kit to determine if ethanol and gasoline fuel blends comply with federal and state laws. A certificate of analysis accompanies each kit.

suitable for D5501 per ASTM

Components

Sol.#1 Ethanol:Heptane: Methanol (92%, 7.40%, 0.60%)
Sol.#2 Ethanol:Heptane: Methanol (93%, 6.50%, 0.50%)
Sol.#3 Ethanol:Heptane: Methanol (94%, 5.60%, 0.40%)
Sol.#4 Ethanol:Heptane: Methanol (95%, 4.70%, 0.30%)
Sol.#5 Ethanol:Heptane: Methanol (96%, 3.80%, 0.20%)
Sol.#6 Ethanol:Heptane: Methanol (97%, 2.90%, 0.10%)
Sol.#7 Ethanol:Heptane: Methanol (98%, 1.95%, 0.05%)

40361-U	1 pkg

Fuel Ethanol Residual Saccharides Mix

Quantitative standard for monitoring unfermented sugars present during the process of transforming corn feedstock into ethanol.

Components

Glycerol 1 % (w/v)
D-(+)-Glucose 2 % (w/v)
Maltotriose (DP_3) 1 % (w/v)
Maltose monohydrate 2 % (w/v)
L-(+)-Lactic acid .3 % (w/v)
Acetic acid .3 % (w/v)
Dextrin 3.25 % (w/v)
Ethanol 12 % (w/v)

48468-U	10 × 2 mL

Ethanol standards 10% (v/v)

Ethanol solution; Ethyl alcohol CH_3CH_2OH
[64-17-5] C_2H_5OH FW 46.07

- **aqueous solution**

Flame-sealed flint ampule

E2385-10AMP	10 amp

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Sigma-Aldrich and GeneGo have integrated Sigma-Aldrich product listings into the MetaCore™ and MetaDrug™ software suites.

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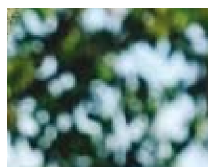


Enzymes and Reagents for BioDiesel Research

Lipases for Transesterification

Amano Lipase PS

- (immobilized on diatomite)

708011-10G	10 g

Amano Lipase PS, from *Burkholderia cepacia*

- activity: ≥30,000 U/g, pH 7.0, 50 °C (Optimum pH and temperature)

534641-10G	10 g
534641-50G	50 g

Amano Lipase PS-C II (immobilized on ceramic)

One unit will produce 1.0 micromole of 1-Phenethyl alcohol to 1-Phenethyl acetate per min. at 25 °C in the presence of vinyl acetate

534889	Inquire

Amano lipase PS-IM (immobilized on diatomaceous earth)

709603-10G	10 g

Amano Lipase PS-C I (immobilized on ceramic)

One unit will produce 1.0 micromole of 1-Phenethyl alcohol to 1-Phenethyl acetate per min. at 25 °C in the presence of vinyl acetate

534897	Inquire

Lipase from *Candida* sp.

- Novozymes® CALB L recombinant, expressed in *Aspergillus niger*

aqueous solution

Minimum 5,000 LU/G of liquid

L3170-50ML	50 mL

Lipase acrylic resin from *Candida antarctica*

Novozym® 435
[9001-62-1]

Immobilized preparation of a thermostable lipase, particularly useful in the synthesis of esters and amides, and has a broad substrate specificity.

Lipase from (B lipase) *Candida antarctica* produced by submerged fermentation of a genetically modified *Aspergillus oryzae* microorganism and adsorbed on a macroporous resin.

- activity: ≥10,000 U/g, recombinant, expressed in *Aspergillus niger*

A product of Novozyme Corp.

L4777-3G	3 g
L4777-10G	10 g

Phospholipase C for Degumming Oils

▼ Phospholipase C

Phosphatidylcholine cholinephosphohydrolase; Lecithinase C; Lipophosphodiesterase I; PC-PLC
[9001-86-9]

Hydrolyzes the phosphate bond on phosphatidylcholine and other glycerophospholipids yielding diacylglycerol; this enzyme will also hydrolyze the phosphate bonds of sphingomyelin, cardiolipin, choline plasmalogen and ceramide phospholipids.

Phospholipase C from *Bacillus cereus*

- activity: ≥200 units/mg protein

Lyophilized powder containing approx. 10% protein. Remainder: trehalose, zinc sulfate, and potassium phosphate

One unit will liberate 1.0 µmole of water soluble organic phosphorus from L-α-phosphatidylcholine per min at pH 7.3 at 37 °C.

P6621-250UN	250 units

Phospholipase C from *Clostridium perfringens (C. welchii)*

One unit will liberate 1.0 µmole of water soluble organic phosphorus from egg yolk L-α-phosphatidylcholine per min at pH 7.3 at 37 °C.

- lyophilized powder, activity: 10–50 units/mg protein

P7633-25UN	25 units
P7633-125UN	125 units
P7633-500UN	500 units

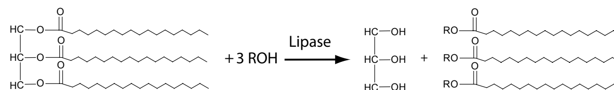
- lyophilized powder, activity: ≥150 units/mg protein

Lyophilized powder in buffered salts

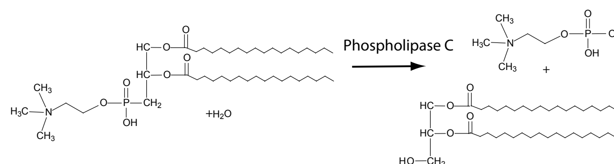
Chromatographically purified

P4039-125UN	125 units
P4039-500UN	500 units

Phospholipase C ▲



Transesterification of triglycerides utilizing commercial lipase preparations is currently under investigation as a low energy input alternative to current "chemical" methods.



Degumming of feedstock oils by hydrolyisis of phospholipids can be catalyzed by phospholipase C. Treatment of phosphatidylethanol, phosphatidylcholine and phosphatidylinositol with phospholipase C enables the hydrolysis and phase separation of the hydrophilic phosphate-containing contaminants.

BioDiesel Analysis

GC Columns for BioDiesel Analysis

MET-Biodiesel

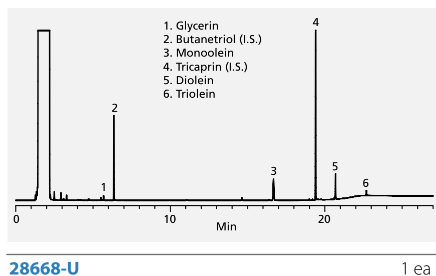
This rugged metal column was designed specifically for the determination of free and total glycerin in B100 biodiesel samples. Features and benefits of this column include:

- Metal is used as the column material, virtually eliminating accidental column breakage during handling
- Metal columns do not require a coating to provide strength (NOTE: the protective polyimide coating on the outside of fused silica columns slowly burns off at 380 °C, exposing bare fused silica which is fragile and susceptible to breakage)
- Includes a guard that both protects the analytical column from excess reagent and non-volatile compounds, extending column life, and acts as a retention gap, minimizing peak broadening
- The guard is an integrated guard, thereby providing protection with a leak-free connection (the guard and analytical column are one continuous piece of tubing; there is no union between the guard and analytical column)
- Low bleed characteristic, even at 380 °C
- Provides good peak shape and resolution for all glyceride impurities of interest
- Able to separate glycerin in addition to mono-/diglycerides (as methyl esters) plus triglycerides from the FAMEs
- A maximum temperature of 380 °C (isothermal) and 430 °C (programmed) exceeds the temperature limitations specified in biodiesel methods such as ASTM D6584 and EN 14105

GC capillary columns

Temp. Limits:

- –60 °C to 380 °C (isothermal)
- –60 °C to 430 °C (programmable)

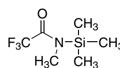


28668-U	1 ea

Derivatization

N-Methyl-*N*-(trimethylsilyl)trifluoroacetamide

MSTFA; *N*-Trimethylsilyl-*N*-methyl trifluoroacetamide
[24589-78-4] $CF_3CON(CH_3)Si(CH_3)_3$
FW 199.25



Silylating agent used to form volatile derivatives for GC-MS analysis.[2]

Improved silylation procedure for simultaneous detection of steroid hormones 17-α-ethynylestradiol and estrone.[1]

Lit cited: 1. *J. Chromatogr.* **1108**, 121 (2006); 2. *Phosphorus, Sulfur Silicon Relat. Elem.* **88**, 53 (1994).

394866-10X1ML	10 × 1 mL
394866-5ML	5 mL
394866-25ML	25 mL

BioDiesel Calibration Standards

Supelco glycerin impurities in biodiesel reference solutions meet the specifications called out in ASTM D6584 and DIN EN 14105. Our reference solutions have been carefully formulated to save 4–6 hours in prep time, minimize the possibility of human error, and eliminate the need to prepare pyridine dilutions. Our standards offer:

- Raw materials tested for identity and purity
- Stock solutions for preparing your own multi-component solutions
- Multilevel, multi-component solutions ready for derivatization
- Monoglyceride commercial mixture for EN14105
- Internal standard solutions
- Certificate of composition shipped with each standard
- Instructions for derivatization with each kit

Our standards are manufactured under the Quality Management System which is registered under ISO 9001:2000.

Biodiesel Calibration Standards

Name	Suitability	Composition	Cat. No.
ASTM® D6584 Individual Standard Solution and Internal Standards Kit	D6584 ASTM	ASTM® D6584 Standard Solution 1 (**Supelco** **44899-U**), 1 mL ASTM® D6584 Standard Solution 2 (**Supelco** **44914-U**), 1 mL ASTM® D6584 Standard Solution 3 (**Supelco** **44915-U**), 1 mL ASTM® D6584 Standard Solution 4 (**Supelco** **44916-U**), 1 mL ASTM® D6584 Standard Solution 5 (**Supelco** **44917-U**), 1 mL ASTM D6584 1,2,4-Butanetriol Solution, Internal Standard #1 (**Supelco** **44896-U**), 5 mL ASTM D6584 Tricaprin Solution, Internal Standard #2 (**Supelco** **44897-U**), 5 mL	**44918-U**
ASTM® D6584 Standard Solution 1	D6584 ASTM	1,3-Diolein, 50 µg/mL Glycerol, 5 µg/mL Monoolein, 100 µg/mL Glyceryl trioleate, 50 µg/mL	**44899-U**
ASTM® D6584 Standard Solution 2	D6584 ASTM	1,3-Diolein, 100 µg/mL Glycerol, 15 µg/mL Monoolein, 250 µg/mL Glyceryl trioleate, 100 µg/mL	**44914-U**
ASTM® D6584 Standard Solution 3	D6584 ASTM	1,3-Diolein, 200 µg/mL Glycerol, 25 µg/mL Monoolein, 500 µg/mL Glyceryl trioleate, 200 µg/mL	**44915-U**
ASTM® D6584 Standard Solution 4	D6584 ASTM	1,3-Diolein, 350 µg/mL Glycerol, 35 µg/mL Monoolein, 750 µg/mL Glyceryl trioleate, 350 µg/mL	**44916-U**
ASTM® D6584 Standard Solution 5	D6584 ASTM	1,3-Diolein, 500 µg/mL Glycerol, 50 µg/mL Monoolein, 1000 µg/mL Glyceryl trioleate, 500 µg/mL	**44917-U**
EN 14105:2003 Monoglyceride Stock Solution	EN 14105 DIN	Monoolein Monopalmitin Monostearin	**49446-U**
EN 14105:2003 Standard Solution 1	EN 14105 DIN	Butanetriol, 80 µg/mL 1,3-Diolein, 50 µg/mL Glycerol, 5 µg/mL Monoolein, 250 µg/mL Tricaprin, 800 µg/mL Triolein, 50 µg/mL	**49441-U**
EN 14105:2003 Standard Solution 2	EN 14105 DIN	Butanetriol, 80 µg/mL Diolein, 200 µg/mL Glycerol, 20 µg/mL Monoolein, 600 µg/mL Tricaprin, 800 µg/mL Triolein, 150 µg/mL	**49442-U**
EN 14105:2003 Standard Solution 3	EN 14105 DIN	Butanetriol, 80 µg/mL Diolein, 350 µg/mL Glycerol, 35 µg/mL Monoolein, 950 µg/mL Tricaprin, 800 µg/mL Triolein, 300 µg/mL	**49443-U**
EN 14105:2003 Standard Solution 4	EN 14105 DIN	Butanetriol, 80 µg/mL Diolein, 500 µg/mL Glycerol, 50 µg/mL Monoolein, 1250 µg/mL Tricaprin, 800 µg/mL Triolein, 400 µg/mL	**49444-U**
EN 14105:2003 Standard Solution Kit	EN 14105 DIN	EN 14105:2003 Standard Solution 1 (**Supelco** **49441-U**), 1 mL EN 14105:2003 Standard Solution 2 (**Supelco** **49442-U**), 1 mL EN 14105:2003 Standard Solution 3 (**Supelco** **49443-U**), 1 mL EN 14105:2003 Standard Solution 4 (**Supelco** **49444-U**), 1 mL	**49445-U**

Karl Fischer Titration—HYDRANAL® Reagents for the Determination of Water in Biodiesel

The presence of water in biodiesel poses problems for a number of reasons. Some of the water is residual from processing while some comes from condensation in the storage tanks. Water content can be determined accurately and reproducibly by Karl Fischer Titration (volumetric, coulometric, and KF-oven methods) using reliable, superior, and proven HYDRANAL reagents (EN 14214).

One-Component Volumetric Reagents

Biodiesel contains components of different chain lengths. Therefore, a solubilizer is often required to dissolve or disperse it in order to extract the moisture. A number of HYDRANAL media are available for effective dispersion of biodiesel. HYDRANAL-Composite 2 is used as the titration agent.

One Component Reagents

Name	Grade	Cat. No.
HYDRANAL®-Composite 1	one-component reagent for volumetric Karl Fischer titration	34827-500ML-R 34827-6X500ML-R 34827-1L-R 34827-6X1L-R
HYDRANAL®-Composite 2	one-component reagent for volumetric Karl Fischer titration	34806-500ML 34806-6X500ML 34806-1L 34806-6X1L 34806-2.5L 34806-4X2.5L
HYDRANAL®-Composite 5	one-component reagent for volumetric Karl Fischer titration	34805-500ML-R 34805-6X500ML-R 34805-1L-R 34805-6X1L-R 34805-2.5L-R 34805-4X2.5L-R
HYDRANAL®-Composite 5 K	one-component reagent for volumetric Karl Fischer titration in ketones and aldehydes	34816-500ML-R 34816-6X500ML-R 34816-1L-R 34816-6X1L-R 34816-2.5L-R 34816-4X2.5L-R
HYDRANAL®-CompoSolver E	–	34734-1L-R 34734-6X1L-R 34734-2.5L-R 34734-4X2.5L-R
HYDRANAL®-KetoSolver	–	34738-500ML-R 34738-6X500ML-R 34738-1L-R 34738-6X1L-R
HYDRANAL®-LipoSolver CM	–	37855-1L 37855-6X1L
HYDRANAL®-LipoSolver MH	–	37856-1L 37856-6X1L
HYDRANAL®-Medium K	–	34698-1L-R 34698-6X1L-R
HYDRANAL®-Methanol dry	–	34741-1L-R 34741-6X1L-R 34741-2.5L-R 34741-4X2.5L-R
HYDRANAL®-Methanol Rapid	–	37817-1L-R 37817-6X1L-R 37817-2.5L-R 37817-4X2.5L-R
HYDRANAL®-Solver (Crude) oil	–	34697-1L-R 34697-6X1L-R 34697-2.5L-R 34697-4X2.5L-R
HYDRANAL®-Working Medium K	–	34817-1L 34817-6X1L
Karl-Fischer reagent	for titrimetric determination of water with one solution	36115-1L 36115-6X1L

Two-Component Volumetric Reagents

Name	Grade	Cat. No.
HYDRANAL®-Solvent	–	34800-1L-R 34800-6X1L-R 34800-2.5L-R 34800-4X2.5L-R
HYDRANAL®-Solvent E	–	34730-500ML-R 34730-6X500ML-R 34730-1L-R 34730-6X1L-R 34730-2.5L-R 34730-4X2.5L-R
HYDRANAL®-Solvent CM	–	34812-1L 34812-6X1L 34812-2.5L 34812-4X2.5L
HYDRANAL®-Solvent Oil	–	34749-1L 34749-6X1L
HYDRANAL®-Titrant 2	–	34811-500ML 34811-6X500ML 34811-1L 34811-6X1L
HYDRANAL®-Titrant 5	–	34801-500ML 34801-6X500ML 34801-1L 34801-6X1L 34801-2.5L 34801-4X2.5L
HYDRANAL®-Titrant 2 E	–	34723-1L-R 34723-6X1L-R
HYDRANAL®-Titrant 5 E	–	34732-100ML-R 34732-500ML-R 34732-6X500ML-R 34732-1L-R 34732-6X1L-R 34732-2.5L-R 34732-4X2.5L-R
Karl-Fischer reagent	for titrimetric determination of water with two separate solutions	36116-1L 36116-6X1L
Karl-Fischer reagent	for titrimetric determination of water with two separate solutions	36117-1L 36117-6X1L

Coulometric Titration Reagents

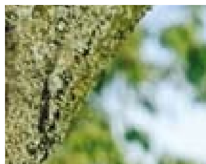
The coulometric procedure is more sensitive than the volumetric titration method. Determination of water is carried out in a coulometric cell (with or without diaphragm). The precision of the coulometric cell and the sample manipulation can be tested by means of HYDRANAL Water Standard 0.10.

Name	Grade	Cat. No.
HYDRANAL®-Coulomat A	anolyte for coulometric Karl Fischer titration (for cells with diaphragm)	34807-500ML 34807-6X500ML
HYDRANAL®-Coulomat E	–	34726-500ML-R 34726-6X500ML-R
HYDRANAL®-Coulomat AD	reagent for coulometric Karl Fischer titration for cells without diaphragm	34810-500ML-R 34810-6X500ML-R
HYDRANAL®-Coulomat AF 7	anolyte for coulometric Karl Fischer titration (suitable for coulometer AF7)	34829-1L-R 34829-6X1L-R
HYDRANAL®-Coulomat AG	anolyte for coulometric Karl Fischer titration. Suitable for cells with and without diaphragm	34836-500ML-R 34836-6X500ML-R 34836-1L-R 34836-6X1L-R
HYDRANAL®-Coulomat AG-H	anolyte for coulometric Karl Fischer titration in long-chained hydrocarbons (free of halogenated hydrocarbons)	34843-500ML 34843-6X500ML
HYDRANAL®-Coulomat AG-Oven	–	34739-500ML-R 34739-6X500ML-R
HYDRANAL®-Coulomat AK	anolyte for coulometric Karl Fischer titration in ketones (for cells with and without diaphragm)	34820-500ML-R 34820-6X500ML-R
HYDRANAL®-Coulomat CG	catholyte for coulometric Karl Fischer titration. Free of halogenated hydrocarbons	34840-50ML-R 34840-6X50ML-R
HYDRANAL®-Coulomat CG-K	–	34821-50ML-R 34821-6X50ML-R
HYDRANAL®-Coulomat Oil	–	34868-100ML-R 34868-6X100ML-R 34868-500ML-R 34868-6X500ML-R

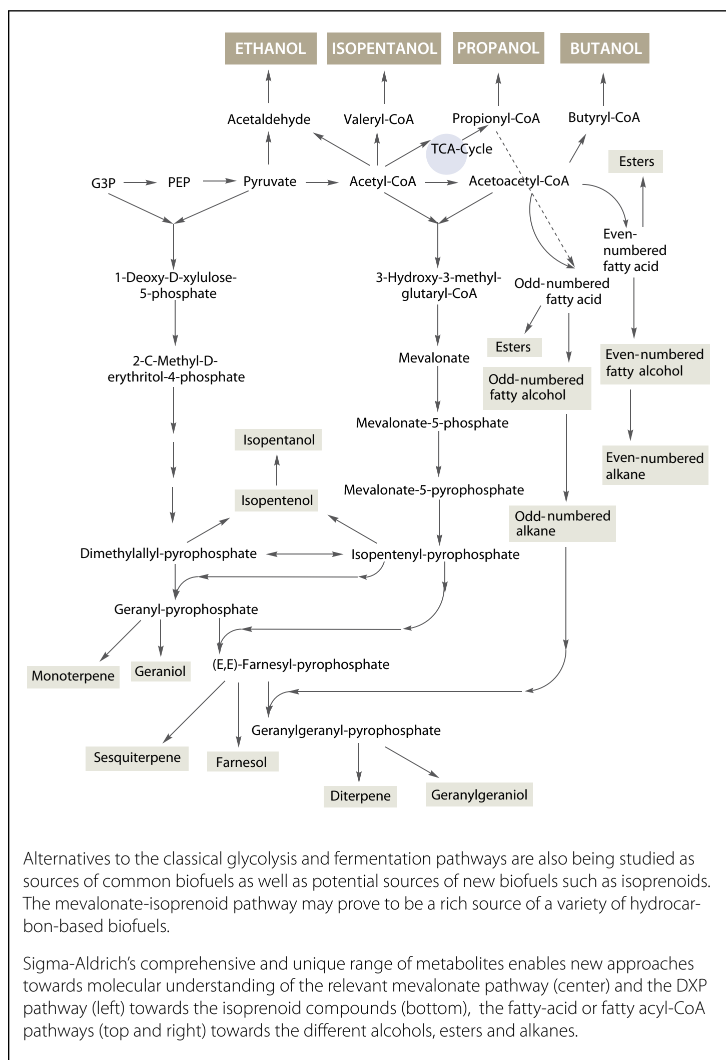
Karl Fischer Oven products

This method is suitable for samples that release water at a higher temperature. In this method, HYDRANAL-Coulomat CG is placed in the cathode chamber and HYDRANAL Coulomat AG Oven is added to the anode chamber of a coulometric cell with diaphragm. A cell without a diaphragm requires only the Coulomat AG Oven. HYDRANAL Coulomat AG-H or HYDRANAL Coulomat AD may be used as a replacement for HYDRANAL Coulomat AG Oven. HYDRANAL Molecular Sieve 0.3 mm is a drying medium for the carrier gas.

Name	Grade	Cat. No.
HYDRANAL®-Coulomat AG-Oven	–	34739-500ML-R 34739-6X500ML-R
HYDRANAL®-Coulomat CG	catholyte for coulometric Karl Fischer titration. Free of halogenated hydrocarbons	34840-50ML-R 34840-6X50ML-R
HYDRANAL®-Molecular sieve 0.3 nm	–	34241-250G-R 34241-1KG-R
HYDRANAL®-Water Standard KF-Oven, 140–160 °C	–	34693-10G-R
HYDRANAL®-Water Standard KF-Oven 220 °C–230 °C	–	34748-10G-R 34748-6X10G-R



Metabolite Standards for Mevalonate and Isoprenoid Pathway Analysis



Alternatives to the classical glycolysis and fermentation pathways are also being studied as sources of common biofuels as well as potential sources of new biofuels such as isoprenoids. The mevalonate-isoprenoid pathway may prove to be a rich source of a variety of hydrocarbon-based biofuels.

Sigma-Aldrich's comprehensive and unique range of metabolites enables new approaches towards molecular understanding of the relevant mevalonate pathway (center) and the DXP pathway (left) towards the isoprenoid compounds (bottom), the fatty-acid or fatty acyl-CoA pathways (top and right) towards the different alcohols, esters and alkanes.

Name	Cat. No.
Acetoacetyl coenzyme A sodium salt hydrate	A1625-5MG A1625-10MG A1625-25MG
Acetyl coenzyme A sodium salt, powder	A2056-1MG A2056-5MG A2056-10MG
Acetyl coenzyme A trilithium salt	A2181-1MG A2181-5MG A2181-10MG
Butyryl coenzyme A lithium salt hydrate	B1508-5MG B1508-10MG B1508-25MG
1-Deoxy-D-xylulose-5-phosphate sodium salt	13368-1MG 13368-5MG
γ,γ-Dimethylallyl pyrophosphate triammonium salt	D4287-1VL D4287-5VL
Farnesyl monophosphate ammonium salt solution, methanol:ammonia solution	F1803-5VL
Farnesyl pyrophosphate ammonium salt solution, methanol:ammonia solution	F6892-1VL F6892-5VL
Geranylgeranyl monophosphate ammonium salt solution, methanol:ammonia solution	G2543-5VL
Geranylgeranyl pyrophosphate ammonium salt solution methanol:ammonia solution	G6025-1VL G6025-5VL
Geranyl monophosphate ammonium salt solution, methanol:ammonia solution	G2293-1VL G2293-5VL
Geranyl pyrophosphate ammonium salt	G6772-1VL G6772-5VL
DL-Glyceraldehyde 3-phosphate solution	G5251-25MG G5251-100MG
DL-3-Hydroxy-3-methylglutaryl coenzyme A sodium salt	H6132-5MG H6132-10MG
Isopentenyl monophosphate ammonium salt solution	I1157
Isopentenyl pyrophosphate triammonium salt solution	I0503-1VL I0503-5VL
(*R*)-Mevalonic acid sodium salt	41288
(±)-Mevalonic acid 5-phosphate trilithium salt hydrate	79849-10MG 79849-50MG
(*R*)-Mevalonic acid 5-pyrophosphate tetralithium salt	77631
(±)-Mevalonic acid 5-pyrophosphate tetralithium salt	94259-10MG 94259-50MG
(*R*)-(–)-Mevalonolactone	68519-100MG
(±)-Mevalonolactone	M4667-1G
Phospho(enol)pyruvic acid monopotassium salt	P7127-100MG
Phospho(enol)pyruvic acid monosodium salt hydrate	P0564-100MG
Phospho(enol)pyruvic acid trisodium salt hydrate	P7002-100MG
n-Propionyl coenzyme A lithium salt	P5397-5MG
Pyruvic acid	107360-25G
Sodium pyruvate	15990-25G

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