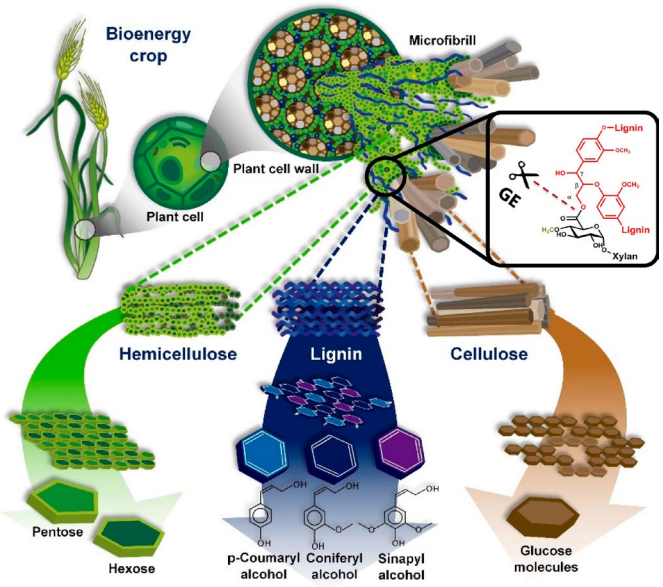
New and unique to Megazyme: Glucuronoyl Esterase Assay Kit (K-GEUX3)

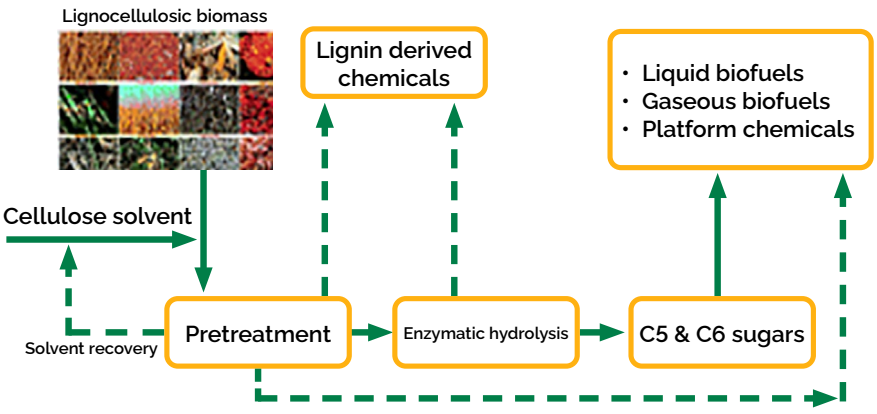
Background: Glucuronoyl Esterase (GE) and its role in valorisation of Lignocellulose.

In a climate of increased global awareness of the importance of renewable resources, biorefineries and industries involved in the manufacturing of bio-based materials are instrumental for the valorisation of biological waste into substitutes of commonly employed oil-based materials.

Lignocellulose is the most abundant biomass on the planet, however its valorisation in **biorefineries** and in the **pulping/paper industry** is problematic and often uneconomic due to the need of harsh, costly and energy demanding pre-treatment processes. Lignin is a recalcitrant material that is difficult to separate from cellulose in an environmentally friendly process.



The use of **enzyme preparations** to treat lignocellulosic bioresources and break down these chemically complex materials into building blocks that can be used to produce bio-based materials is an environmentally friendly and well-established practice. Thanks to their specialised and sophisticated machinery, enzymes can delignify and depolymerize lignocellulose under mild conditions and without the need for solvents, high temperatures and the use of caustic chemicals.



New enzyme discovery is an important line of research both in **academia** and in the **enzyme manufacturing industry.** The R&D pursuit of the study of activity exerted by new enzymes and their correct characterisation is essential for the development of new industrial enzyme preparations to be used in the processing of lignocellulosic materials by biorefineries and the pulping/paper industries.

Among the enzymes that are known to be helpful for the treatment of lignocellulosic material, **glucuronoyl esterases (GE)** are a relatively novel class of enzymes that have been demonstrated to have a role in the delignification process. More precisely, GEs are responsible to promote the hydrolysis of the bonds which link the sugars in hemicellulose to the polyphenols present in lignin thereby improving delignification under mild conditions. Existing industrial enzyme preparations have been found to contain levels of GE which can be exploited for the valorisation of lignocellulose material by biorefineries, pulping and paper industries.

Why is there a need for Megazyme's new Glucuronoyl Esterase Assay Kit (K-GEUX3)

GE is a relatively new class of enzymes first described in 2006, practical diagnostic tools allowing for GE characterisation and quantitative activity measurement have not been commercially available
This has hampered new GE discovery and the development of novel industrial enzyme preparations which can handle lignocellulosic material more efficiently. Some methods have been reported that allow for the activity measurement of GE. These however are either not quantitative, or not commercially available and in general not suited for automation, a key requisite for industrial enzyme discovery.

The gap for commercially available diagnostic tools for the study of GE has now been filled by the release of the new **Glucuronoyl Esterase Assay Kit (K-GEUX3)** by Megazyme. This kit is a unique product which allows selective and accurate measurement of the activity of GE even when present as a component of industrial enzyme cocktails. **The K-GEUX3 assay format allows for a quantitative, rapid, accurate and user-friendly method** for the analysis of GE activity which is suitable for auto-analysers and high throughput screening.

About Glucuronoyl Esterase Assay Kit (K-GEUX3)

K-GEUX3 assay kit is positioned towards two main applications:

1) **GE discovery and development**
   For the identification of novel and improved GE variants within academia and industry

2) **QC of commercial enzyme cocktails**
   To allow enzyme manufacturers to easily standardise their lignocellulosic degrading enzyme product batches for GE activity.

The Megazyme K-GEUX3 method has significant advantages over other methods described by researchers. K-GEUX3 is the only commercially available diagnostic tool suitable for quantitative measurement of GE activity, that it is suitable for the measurement of crude enzyme preparations and is also compatible with auto-analysers.

About Glucuronoyl Esterase Assay Kit (K-GEUX3)

Area	Application
Research	GE studies at academic level is a relatively new research line pursued by selected universities However, GE has become more prominent in the past couple of years, as evidenced by an increase in publication. Academic research in GE is mainly focused at discovering new GE enzymes from new sources and characterise them. GE are part of those enzymes that can be used in the field of renewable bioresources and 'green' R&D which are a 'hot topic' and favoured among sponsors who provide financial support and grants to academic researcher.
Enzyme Manufacturing and Biotechnology	Enzyme manufacturers in the business of bio-based materials which produce enzyme cocktails should be interested in measuring GE activity in their existing products for QC purposes. Considering the usefulness of GE in delignification, enzyme manufacturers should also be interested in developing new enzyme cocktails with enhanced GE activities for use by customers who handle lignocellulose materials.
Biorefineries	While it is unlikely that biorefineries will measure GE activity themselves, those biorefineries that handle lignocellulose will be interested in knowing about the benefit of having enzyme cocktails that contain enhanced GE activity, to save on the costly and energy-consuming chemical pre-treatments. As target customers of enzyme manufacturers, biorefineries might be influential in requesting novel enzyme cocktails that contain higher GE activity in order to reduce their costs and optimise their production processes.
Pulp and paper industry	Same as biorefineries above.

Advantages of K-GEUX3:	
Sensitive	Unlike other commercially available substrates, the bespoke structure of the substrate in the GEUX3 kit contains features like the natural GE substrate, this allows for increased sensitivity.
Selective	The bespoke structure of the substrate also makes it selective for the detection of GE even in the presence of other enzymes as is the case in commercial preparations.
Quantitative measurement	Unlike other methods described, the K-GEUX3 format is quantitative and does not require calibration curves.
Commercially available all-in-one box	Unlike other methods described, the K-GEUX3 is the only product for GE that is commercially available and at the same time allows the measurement of GE activity without the need to buy multiple products.
High throughput	The method is automatable and therefore allows for high sample throughput.
Presence of a control enzyme standard	The kit comes with an enzyme standard of known activity which can be used as a control.
No need for HPLC	Unlike other methods, no HPLC is required.
Faster	With a shorter test time than traditional molybdenum method. Total reaction time of approximately 25 mins versus > 60 mins.
No co-solvent needed	Unlike other methods, the substrate is fully water soluble and does not require the use of flammable/hazardous co-solvents which can affect the performance of GE and require dedicated waste disposal.
Simpler	Uses a common UV spectrophotometer and inexpensive plasticware. No standard curve required during analysis.