



General enzyme immobilization for biocatalysis applications

Enzyme immobilization is a requirement for efficient biocatalytic process design. EnginZyme has developed a material for general immobilization, which offers purified/enriched enzyme on an inert and robust support, in a single step from cell lysate. The resulting heterogeneous biocatalyst is reusable for batch reactions in aqueous and organic media, and perfectly suited for continuous reactions (flow chemistry).

Controlled porosity glass (CPG) particles offer excellent flow through properties due to its interconnecting pore structure and incompressible/ non-swelling nature, and is suitable for use in organic solvent and aqueous environments. The hydrophilic porous glass surface is covered with an organic polymer to achieve new surface properties; hybrid CPG (hybCPG™) maintains the benefits of conventional CPG while the surface can be tailored to suit the application. We collaborate with the specialists at Prime Synthesis, Inc. who manufacture the porous solid support materials (CPG and hybCPG™).

EziG is CPG or hybCPG particles modified to bind protein affinity tags.¹ The result is a combined purification and immobilization method based on a porous material which is tailored for the application in question, suitable for any biocatalytic process or purification procedure.

The current products contain chelated Fe(III) for His-tag binding. We can also use Co(II), which gives higher specificity for the His-tag. This can be a benefit when the target enzyme is poorly expressed. The non-toxic Fe(III) gives higher affinity to enable extensive reuse. EziG made from CPG has a hydrophilic surface (EziG 1), from hybCPG with polyvinyl benzyl chloride has a hydrophobic surface (EziG 2) or from hybCPG with a blended co-polymer for a somewhat hydrophilic surface (EziG 3). The material has a narrow pore size distribution, produced with a pore diameter of ~500 Å as standard; which in the case of EziG 2 and 3 leaves an effective pore diameter of ~300 Å due to the polymer coating. A mass loading of 15 - 60% active enzyme is expected. The method has been verified with several enzymes from different enzyme classes, with and without cofactor dependency, cascade reactions, continuous systems and two-phase reactions.

¹ Engelmark Cassimjee K, Kadow M, Wikmark Y, Svedendahl Humble M, Rothstein ML, Rothstein DM, Bäckvall JE. 2014 Chem Commun (Camb). 50:9134-9137

Specifications

Material:	All EziG products are based on controlled porosity glass (CPG).
Surface:	EziG 1: hydrophilic (glass) EziG 2: hydrophobic (polymer) EziG 3: semi- hydrophilic (polymer)
Pore diameter:	EziG 1: 500 +/- 50 Å EziG 2: 300 +/- 50 Å EziG 3: 300 +/- 50 Å
Pore volume:	~1.8 mL/g.
Bulk density:	EziG 1: 0.25 – 0.32 g/mL EziG 2: 0.21 – 0.25 g/mL EziG 3: 0.21 – 0.25 g/mL
Particle size:	120 – 200 mesh (75 – 125 µm)
Chelated Fe³⁺:	>10 µmol/g
pH range:	5 – 10
Optimum pH:	7 – 9