

IGNITOR™

amino release matrix

A PROPRIETARY ENZYME BLEND DESIGNED AND OPTIMIZED TO EFFICIENTLY RELEASE BCAAS AND GLUTAMINE, PROMOTING HEIGHTENED mTOR ACTIVATION –FOR BETTER PROTEIN SYNTHESIS AND SKELETAL MUSCLE GROWTH.

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ABSTRACT:

Protein is a coveted supplement for bodybuilders and serious athletes. However, these consumers do not always reap the full benefits of the substantial amounts of protein they often ingest—typically in sports nutrition—due to limits in the way the human body naturally digests and absorbs protein.

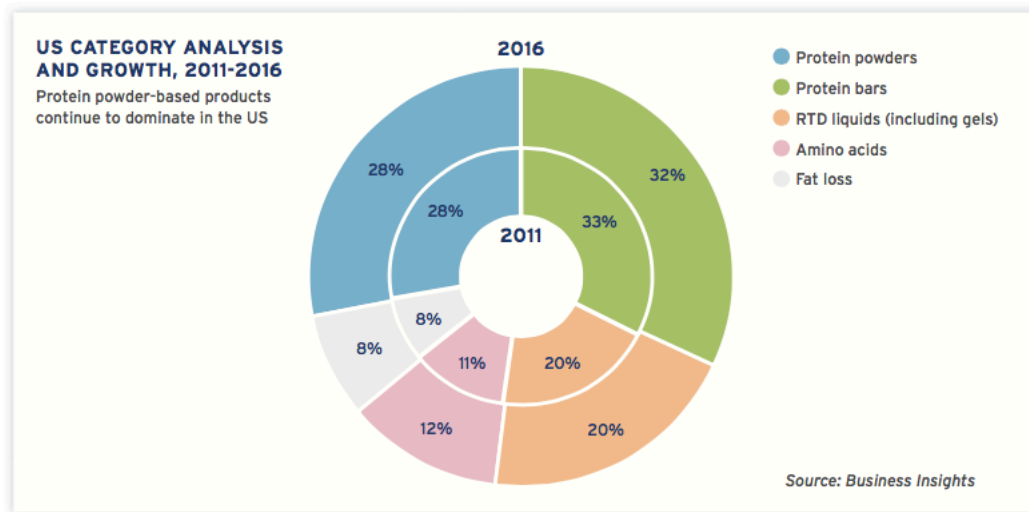
A new, innovative ingredient from Glanbia Nutritionals, IGNITOR™ amino release matrix helps overcome these hurdles. The addition of the IGNITOR™ to protein products promotes greater branch chain amino acid (BCAA) and glutamine release and bioavailability, which subsequently increases the plasma concentration of these amino acids. The resulting mTOR activation helps optimize protein synthesis and ultimately skeletal muscle growth.

IGNITOR™ is also highly beneficial for manufacturers in their quest to improve existing products as well as formulate the next generation of in-demand sports nutrition products, as it requires less protein volume for the same BCAA and glutamine effectiveness, promotes a more efficient protein breakdown for less ingested protein wasted, and comes in a high-quality powder blend suitable for inclusion in RTM, capsule and tablet applications.

INTRODUCTION

In the world of sports nutrition, protein is king, and for good reason. Protein performs a variety of functions in the human body, helping to:

- > Increase muscle mass¹⁻³
- > Prevent a catabolic state during prolonged exercise⁴⁻⁵
- > Enhance nitrogen retention to increase an anabolic state⁶⁻⁷
- > Promote the resynthesis of muscle glycogen following exercise⁸⁻⁹



THE IMPORTANCE OF BRANCH CHAIN AMINO ACIDS

Protein is made up of long chains of amino acids called polypeptides. Once ingested, the protein is broken down into smaller and smaller chains of amino acids and eventually into the individual amino acids themselves, which are then absorbed into the body for use in a variety of functions, including building hormones and neurotransmitters, and also promoting immune function plus tissue repair.¹⁰⁻¹³ Some of the most important amino acids for skeletal muscle growth, and thus sports nutrition, are the BCAAs leucine, valine and isoleucine, so called because of their branched side groups.¹⁴⁻¹⁵

LEUCINE AND THE mTOR PATHWAY

Leucine is considered the most important BCAA for sports nutrition because it has been shown to activate the mTOR^A pathway, resulting in increased muscle development with exercise, as well as reduced muscle damage.¹⁶⁻¹⁸ mTOR is an enzyme in its own right. When it becomes phosphorylated it activates a signal transduction pathway that ultimately stimulates muscle protein synthesis and muscle hypertrophy, or increase in muscle size.^{17,19}

CLINICAL PROOF: LEUCINE PROMOTES SKELETAL MUSCLE GROWTH

Increased leucine, glucose and phosphatidic acid concentrations all activate mTOR, which subsequently activates protein synthesis and increases a cell's capacity to produce new proteins.¹⁹⁻²¹ Ingestion of leucine independent of exercise has been shown to increase the phosphorylation of mTOR (mTOR activation) in the skeletal muscle of rats.¹⁶ In fact, animals given 1.35 g/kg body weight leucine—the equivalent of 0.22 grams/kg in humans or 22 grams in a 220-pound weightlifter—showed a three-fold increase in mTOR activation, demonstrating that ingestion of leucine activates mTOR. A separate animal study further proved that mTOR was responsible for skeletal muscle growth.²² Here, the hind limbs of wild-type and heterozygous mTOR mice^B were put in a cast for seven days to produce muscular atrophy. Muscle regrowth was measured for 20 days after cast removal to determine the effect of mTOR on stimulating skeletal muscle growth. Ultimately, mTOR-heterozygous mice showed a significantly decreased rate of muscle synthesis following atrophy, taking 20 days to fully recover compared to 10 days for the wild-type mice.

It was shown that leucine supplementation rescued muscle strength following muscle damage, demonstrating that leucine supplementation assists with increased skeletal muscle growth and healing.

A similar experiment was performed to ascertain leucine's role in muscle growth and regeneration. In this instance, muscle tissue was damaged via cryolesion administration.²³ Muscle regeneration^C was then measured with and without leucine supplementation at a rate of 1.35 g/kg per day.

Results showed that leucine significantly increased myofiber cross-sectional area, both in the absence and presence of damage via cryolesion. Leucine supplementation also rescued muscle strength following exercise, demonstrating that leucine supplementation assists with increased skeletal muscle growth and healing.

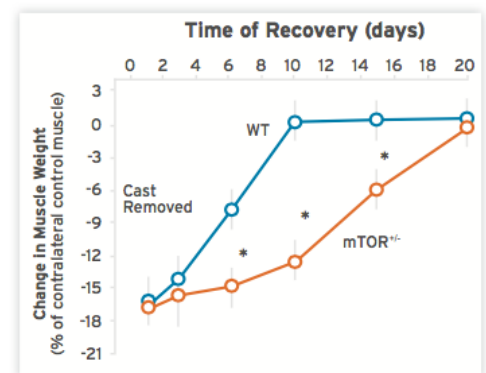
^AMammalian Target of Rapamycin.

^BHeterozygous mTOR mice have reduced mTOR activity.

^CAs measured via myofiber cross-sectional area.

WHAT MAKES IGNITOR™ A PREFERRED SPORTS NUTRITION INGREDIENT

- > More efficient release of BCAAs and glutamine
- > Less protein volume required for the same BCAA and glutamine effectiveness
- > More efficient protein breakdown, less ingested protein wasted
- > Powdered enzyme blend suitable for inclusion in RTM, capsule and tablet applications
- > GRAS
- > Made in the USA
- > Unrivaled Glanbia Nutritionals quality



Reduced mTOR activity results in a decreased rate of muscle synthesis following atrophy.

From *J Physiol* 591.11; 2911-2923 (2013)



THE SYNERGIES OF PROTEIN INTAKE, MTOR ACTIVATION AND RESISTANCE TRAINING

Ingestion of protein in conjunction with resistance exercise (RE) has also been shown to increase the phosphorylation of mTOR, or mTOR activation.¹⁷ In this case, men taking 15 grams of whey protein isolate before doing 5 x 10 repetitions of leg presses showed significantly more mTOR activation compared to the placebo group, demonstrating that the leucine in the protein is released and subsequently activates mTOR.

Muscle fiber size and muscle thickness was also measured in this study, with the protein treatment group gaining significantly more of both.¹⁷ This proves that ingestion of leucine-source protein in conjunction with resistance exercise activates mTOR, leading to increased muscle synthesis and hypertrophy.

Men taking 15 grams of whey protein isolate before leg presses showed significantly more mTOR activation demonstrating that the leucine in the protein is released and subsequently activates mTOR.

THE ROLES OF ISOLEUCINE, VALINE AND GLUTAMINE

While isoleucine, valine and glutamine do not directly stimulate mTOR, they play important indirect roles that assist with skeletal muscle growth. Isoleucine stimulates uptake of glucose by muscle cells, indirectly stimulating mTOR, while valine stimulates uptake of lipids by cells, eventually stimulating mTOR indirectly through phosphatidic acid.²⁴⁻²⁶ Glutamine stimulates the transport of leucine into cells, thereby increasing its availability.²⁷⁻²⁸ Glutamine also accounts for ~61% of skeletal muscle tissue, making it an integral building block for muscle growth.

WHEY PROTEIN: PREEMINENT IN SPORTS NUTRITION

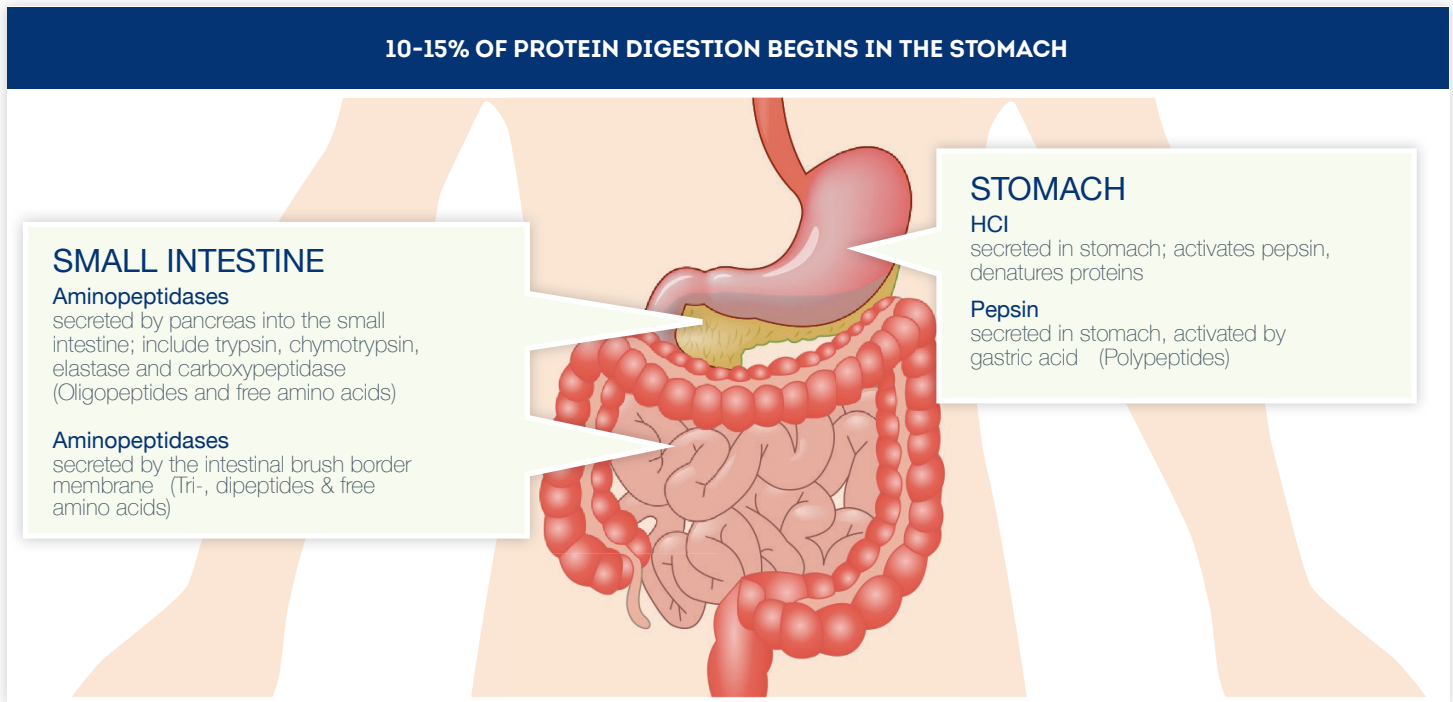
BCAAs are metabolized mainly in skeletal muscle, unlike the other 17 amino acids which must first pass through the liver before they are available to muscle tissue.²⁹⁻³⁰ This is important to remember, because protein powders that contain higher concentrations of BCAAs have a much higher bioavailability.

Since BCAAs comprise a large percentage of whey protein—up to 30%—whey protein has become the gold standard for sports nutrition protein products. Unfortunately, the human body cannot efficiently break down the polypeptides in this substance, especially when taken in large quantities like those seen in a typical sports nutrition product. Due to the digestive process and transit time of food through the digestive system, on average less than 40% of the whey protein we ingest is actually absorbed.

The key to producing better absorption of protein in the body is the introduction of enzymes.

THE ESSENTIAL CATALYTIC EFFECTS OF ENZYMES

Enzymes are actually globular proteins that act as catalysts to accelerate specific reactions. For example, the enzyme lipase accelerates the breakdown of lipids, while the enzyme lactase accelerates the breakdown of lactose.



Each enzyme has a unique catalytic site that acts on a specific substrate, much like a lock and key. A lock will not open unless the key fits perfectly and all the tumblers fall into place.

The primary role of an enzyme is to optimize the efficiency of a reaction, which can be accomplished by:

- > Increasing the rate of the reaction
- > Lowering the energy required for the reaction to occur
- > Reducing the time needed to complete the reaction

In the case of digesting protein, native enzymes such as the protease pepsin, along with pancreatin^D help cleave the bonds between amino acids to assist in breaking down the protein. In the stomach, pepsin and acid work in harmony to first denature the proteins, then help reduce them into long amino acid chains called polypeptides.

In the small intestine, aminopeptidases secreted by the pancreas—trypsin, chymotrypsin, elastase and carboxypeptidase—further reduce these polypeptides into smaller chains of 10,000 amino acids or less. The farther along the small intestine these amino acid chains travel, the shorter they become, until the majority consist of tripeptides, dipeptides and free amino acids—all of which are absorbed for use in the body.

The key to producing more efficient breakdown and better absorption of protein in the body is the introduction of enzymes. IGNITOR™ from Glanbia Nutritional is uniquely formulated to optimize those critical enzymes.

^DA mixture of proteases, lipases and amylases.

ISSUES ASSOCIATED WITH SUBSTANTIAL PROTEIN INTAKE

The endogenous enzymes created by the body during the digestive process work efficiently, but do not have a lot of time to take effect before the protein moves farther down the digestive tract. This is especially true in cases where a large amount of protein has been ingested in one sitting, say from a protein shake after a workout. In cases like this the endogenous enzymes cannot fully digest the proteins and release amino acids such as the coveted BCAAs.³¹

Undigested or partially digested protein in the small intestine presents a couple of problems for athletes, particularly those that favor protein supplements—notably bodybuilders. First, their bodies are not receiving all the benefits of the protein they have ingested, since much of the BCAAs are still locked up in oligopeptides and will go to waste. Second, bacteria farther down the digestive tract will feed on these undigested proteins, petrifying them and releasing byproducts which can cause gas, cramping and bloating.

IGNITOR™ amino release matrix is a proprietary blend of proteolytic enzymes designed and optimized to specifically release the BCAAs and glutamine from whey protein.

Because of this, many ingredient manufacturers choose to use hydrolyzed protein in their products, providing protein that is already broken down into smaller components for better absorption. However, along with being more expensive, hydrolyzed protein can also have undesirable odor and flavor profiles that may be difficult to mask. The solution to both of these problems is the efficient breakdown of protein via digestive enzyme optimization.

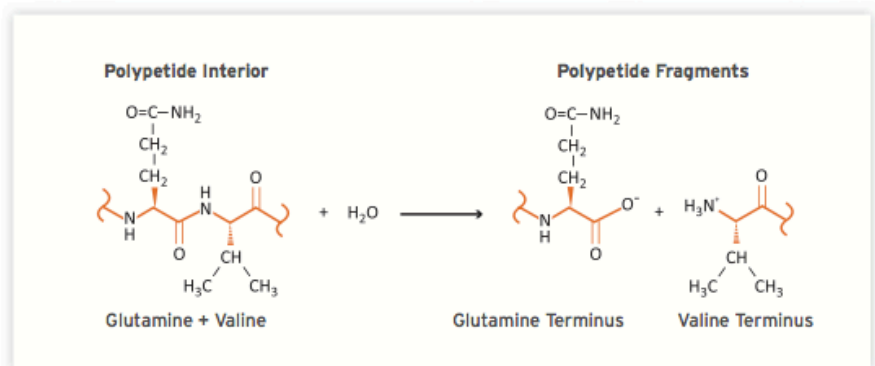
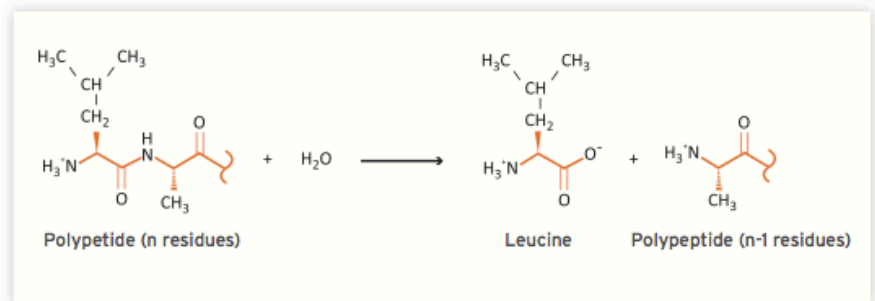
IGNITOR™ from Glanbia Nutritionals is uniquely formulated to provide these optimized enzymes, focusing on the release of the amino acids that matter most to sports nutrition consumers.

IGNITOR™ ACTIVATES THE RELEASE OF BCAAS

IGNITOR™ amino release matrix is a proprietary blend of proteolytic enzymes designed and optimized to specifically release the BCAAs and glutamine from whey protein. It is composed of two different types of enzymes: endopeptidases and exopeptidases.

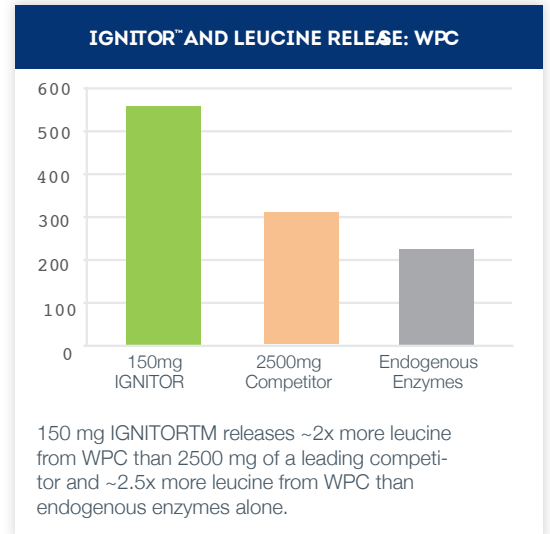
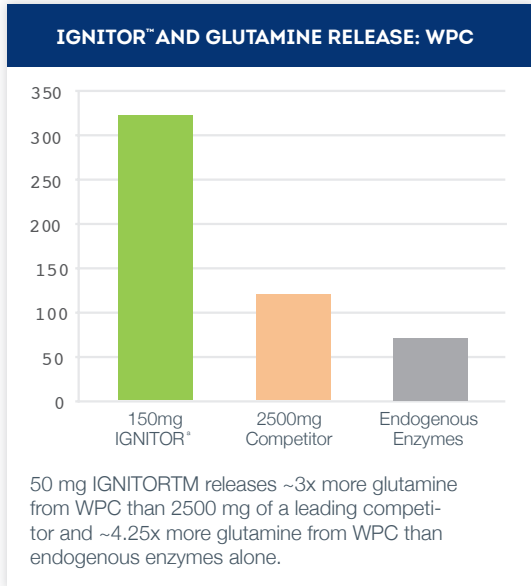
Endopeptidases hydrolyze the protein molecule at the interior peptide bonds, liberating smaller peptides, while exopeptidases hydrolyze the protein molecule at the terminus of the peptide chain, liberating an individual amino acid.

The exopeptidases in IGNITOR™ have been optimized to specifically cleave off the BCAAs and glutamine; while the endopeptidases in the product have been optimized to specifically cleave next to leucine, valine, isoleucine and glutamine. This creates fragments with the desired amino acids at the end, allowing the exopeptidases to cleave them off more easily, resulting in a much higher proportion of individual branch chain amino acids and glutamine being released from the protein. Other enzyme blends on the market are general endo- and exopeptidases, meaning that they do not specifically target the release of BCAAs or glutamine.



STUDIES PROVE IGNITOR™ IS EFFECTIVE

In a simulated gastrointestinal study conducted at body temperature, a USP dissolution was used for pharmacokinetic evaluations and comparisons to determine the efficacy of IGNITOR™ in releasing BCAAs and glutamine from whey protein concentrate (WPC).³² The study included both simulated gastric and intestinal conditions, and utilized 40 grams of WPC as the protein source. A mixture of the endogenous gastrointestinal enzymes pepsin and pancreatin was used as the control, and included in all samples. 150 mg of IGNITOR™^E was tested against 2500 mg of a competing general enzyme blend, and free amino acids were assayed using HPLC to determine their degree of release from the WPC.



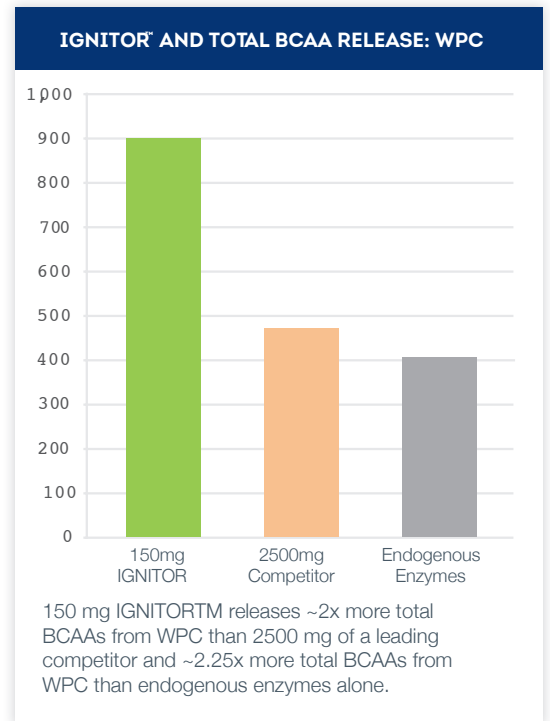
The results of the study were compelling. Indeed, it was found that 150 mg of IGNITOR™ releases ~2x more leucine from WPC than 2500 mg of a leading competitor and ~2.5x more leucine from WPC than endogenous enzymes alone. Similarly, IGNITOR™ released ~2x and 2.25x more total BCAAs respectively than the competing blend and endogenous enzymes alone.

Finally the study showed that glutamine release was ~3x and 4.25x higher with IGNITOR™, respectively. A similar study using whey protein isolate (WPI) produced comparable results, with 100 mg of IGNITOR™ releasing 4x more leucine, 3.25x more total BCAAs, and 2.25x more glutamine than endogenous enzymes alone.

The results of the two studies demonstrate that IGNITOR's™ specially designed blend of proteolytic enzymes delivers on its promise to optimize the release of BCAAs and glutamine from WPC.

To further validate IGNITOR's™ superior performance, Glanbia Nutritionals plans to study further.

^E 75 mg per 20 g of whey protein concentrate.



CONCLUSION: IGNITOR™ PROMOTES SUPERIOR PROTEIN SYNTHESIS AND SKELETAL MUSCLE GROWTH

The addition of the IGNITOR™ amino release matrix to protein helps bring about greater BCAA and glutamine release and bioavailability, which increases the plasma concentration of these amino acids. The effect of this is heightened mTOR activation, which can lead to protein synthesis and ultimately skeletal muscle growth.

IGNITOR™ also provides a number of important benefits for manufacturers striving to succeed in today's lucrative (and highly competitive) sports nutrition market by including this ingredient in ready-to-mix drink mixes, meal replacements and sports formulas. It offers a more efficient release of BCAAs and glutamine, requires less protein volume for the same BCAA and glutamine effectiveness, has GRAS status and also comes in a high-quality enzyme powder blend that is made in the U.S.A. As a result, it is ideal for the development of innovative sports nutrition products targeting bodybuilders and serious athletes.

WHY GLANBIA NUTRITIONALS

Glanbia Nutritionals is a premier provider of ingredient solutions including customized premixes and nutritional ingredients for the food, beverage and supplement industries. Look to us for:

- > **AGILITY:** Solutions customized to meet your individual, complex and evolving needs
- > **QUALITY:** Careful adherence to standards of excellence
- > **SERVICE:** Passionate loyalty to your company and products

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