

1 Product card	<u>ImunoCare</u>
<u>Fast-action holistic immuno-booster with high purity of yeast-derived essential nucleotides</u>	
<p style="text-align: center;">Short description:</p> <p>Fast-action holistic immuno-booster based on 4 yeast-derived nucleotides. 60% content of Adenine, Cytosine, Guanine and Uracil. Yeast-derived nucleotides boost the natural immune system by providing building blocks for cell division of macrophages, activating macrophages and phagocytes, reinforcing the gut barrier, and through antioxidation. Vitamin C is a powerful antioxidant and activator of immune cells participating in their signalling pathways. Zinc has antioxidant effects and activates T cells, crucial immune cells that trigger specific and adaptive response.</p>	
<p>Ingredients:</p> <ol style="list-style-type: none"> 1. Imunell™ – yeast extract with high purity content of 4 essential nucleotides (60%) 	<ol style="list-style-type: none"> 2. Vitamin C. 3. Zinc.
<p>Problem statement:</p> <p>80% of immune cells of the human body are located in the intestines so the immune and gastrointestinal systems are interrelated.¹ Both systems constantly adapt to outside, alien compounds. After the skin, intestine is the main natural barrier against external factors.</p> <p>In the gut, there is an intricate interplay between the intestinal microbiota, the intestinal epithelial layer, the local mucosal immune system, and the systemic immune system.² These systems thus have a faster cell replacement and a higher cellular turnover in order to provide innate and adaptive immune response.</p> <p>Nucleotide supplementation has been long recognized as very effective for fast and effective immune system activation. Nucleotides can activate immune response in 30 min to 2 hours.</p> <p>The most commonly used nucleotide supplementation are various types of extracts from dairy cow colostrum. Colostrum, or first milk, is the first form of milk produced by the mammary glands of mammals (including humans).</p> <p>Bovine- and animal-derived colostrum is not suitable for vegans and vegetarians and presents risks of transmitting diseases, such as BSE (mad cow disease).</p> <p>The quality, amount and composition of nucleotide supplements also varies.</p>	
<p>Intended use:</p> <p>Supplementation for complete activation of the immune system on molecular and systemic levels.</p>	

¹ Wiertsema, S. P., van Bergenhenegouwen, J., Garssen, J., Knippels, L. M. J., The Interplay between the Gut Microbiome and the Immune System in the Context of Infectious Diseases throughout Life and the Role of Nutrition in Optimizing Treatment Strategies, *Nutrients*, Vol. 13, Issue 3 (2021), art. 886.

² Wiertsema, S. P., van Bergenhenegouwen, J., Garssen, J., Knippels, L. M. J., The Interplay between the Gut Microbiome and the Immune System in the Context of Infectious Diseases throughout Life and the Role of Nutrition in Optimizing Treatment Strategies, *Nutrients*, Vol. 13, Issue 3 (2021), art. 886.

Benefits:

1. Stimulates quick immune system reaction within 30 min to 2 h
2. Activates the immune cells (T cells, macrophages and phagocytes)
3. Enhances phagocytosis so that the body attacks external invading factors
4. Stimulates antioxidation on several levels and through different cellular mechanisms.
5. Reinforces the gut barrier and acts as prebiotic (intestinal barrier to external factors)
6. Supports cellular regeneration by providing nucleotides (4 essential compounds in the cellular nucleus) as nutrients for faster cell division processes in the gastrointestinal and immune systems

Main target population:

1. Adult women and men with compromised immunity.
2. Preventive and curative supplementation against viral and bacterial infections during autumn and winter times.

2. Explanation of immune system mechanisms:

2.1 Interrelated immune and gastrointestinal systems

Immune and gastrointestinal systems constantly adapt to outside, alien compounds. After the skin, intestine is the main natural barrier against external factors. Immune and gastrointestinal systems are interrelated and together contribute to immune protection. 70-80% of immune cells are found in the gut, and there is an intricate interplay between the intestinal microbiota, the intestinal epithelial layer, the local mucosal immune system, and the systemic immune system.³

2.2 High cell replacement and turnover in GI and immune systems requires nucleotides

In order to provide quick innate and adaptive immune response, the two systems have a faster cell renewal and replacement, and a higher cellular turnover. To build DNA and RNA for cell division, cells require their building blocks, nucleotides.

2.3 The gut barrier – effective physical and functional barrier to antigens

After gastric juice and pancreatic enzymes, which both have antibacterial properties and participate in the luminal integrity of the gut barrier, decompose ingested food, the gut barrier functions as effective response to alien compounds presenting danger for the homeostasis of the body.

The gut barrier is a functional unit made up of two main components:

- a **physical barrier** surface, which prevents bacterial adhesion and regulates paracellular diffusion to the host tissues, and
- a deep **functional barrier**, that is able to discriminate between pathogens and commensal microorganisms, organizing the immune tolerance and the immune response to pathogens.

The two components are in fact a multi-layer system:

1. **Gut microbiota** are the first physical barrier. Bacteria in the gut are either helpful or pathogenic. Both compete to gain space and energy resources. Healthy bacteria process the molecules necessary to mucosal integrity and modulate the immunological activity of deep barrier. Pre-, pro-, and post-biotics are substances that affect the competition between the two types of bacteria and promote the healthy bacteria.

³ Wiertsema, S. P., van Bergenhenegouwen, J., Garssen, J., Knippels, L. M. J., The Interplay between the Gut Microbiome and the Immune System in the Context of Infectious Diseases throughout Life and the Role of Nutrition in Optimizing Treatment Strategies, *Nutrients*, Vol. 13, Issue 3 (2021), art. 886.

2. **Mucus** is the second physical barrier separating the intraluminal content (inside the gastrointestinal tube) from more internal layers. Mucus is a hydrated network of polymers including proteins (namely the glycosylated mucin proteins) that:
 - a. are essential nutrients for the molecular functioning of gut bacteria and immune cells
 - b. contain antimicrobial products and secretory immunoglobulin A (IgA), principal antibodies secreted as the first line of molecular immune defense.
 - c. polymeric immunoglobulin A (pIgA) is produced by mucosal B cells and transported to the mucosal surface, where it binds on antigens. By binding, they mirror-copy the antigen and
 - i. either neutralize the antigens or
 - ii. transport the transcription information about what other antibodies have to be produced, thereby ensuring immune system mediation and coordination.
3. **Epithelial cells** present a physical and functional barrier.

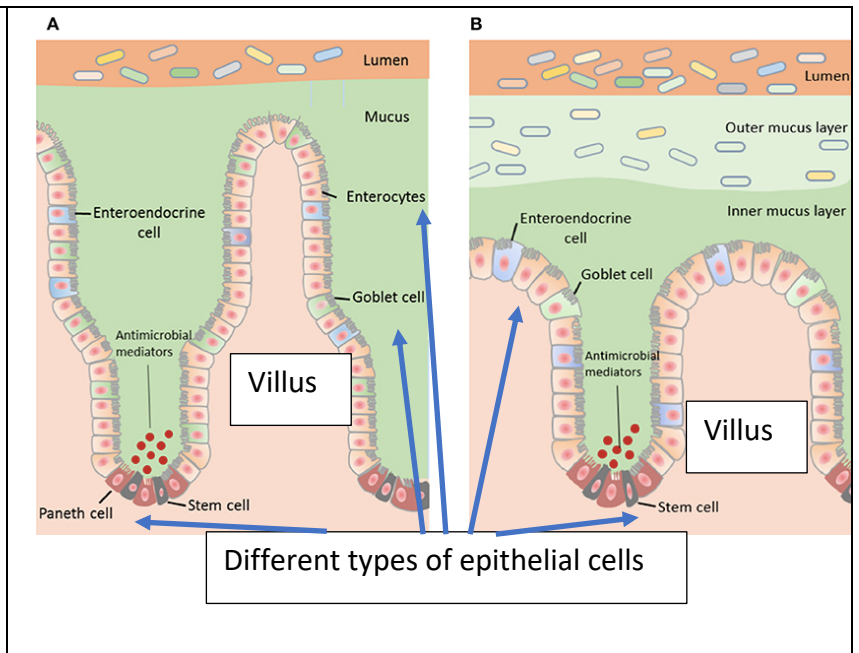
Epithelial cells represent the large majority of cells on the villi, which are numerous finger-like structures protruding into the lumen from the intestine's wall.

A villus is covered with them and thus ensures absorption of nutrients in the small and large intestines.

Epithelial cells are therefore a physical barrier, as a line of connected cells, and a functional barrier, inasmuch as they decompose food and

The **small intestine has the fastest cell turnover in the body.**

Epithelial cells are renewed every 3-5 days by repeating cycles of cell proliferation, migration, and shedding.⁴



4. **The innate and adaptive immune cells** are a functional barrier forming the gut-associated lymphoid tissue (which is responsible for antigen sampling and immune responses).⁵ Innate and adaptive immunity are not mutually exclusive mechanisms of host defense, but rather are complementary, with defects in either system resulting in host vulnerability or inappropriate responses.⁶

Innate immunity:

- Is a **rapid response** activated in a matter of minutes or hours
- Composed of molecules and cells already existing in the body that **directly attack, neutralize or destroy pathogens.**

⁴ Darwich, A.S., Aslam, U., et al., Rostami-Hodjegan, A., Meta-analysis of the turnover of intestinal epithelia in preclinical animal species and humans, *Drug Metabolism and Disposition*, Vol. 42 (2014), pp. 2016-2022.

⁵ Viggiano, D., Ianaro, G., Vanella, G., Bibbò, S., Bruno, G., Simeone, G., Mele, G., Gut barrier in health and disease: focus on childhood, *European Review of Medical Pharmacology*, Vol. 19, Issue 6 (2015), pp. 1077-1185.

⁶ Marshall, J. S., Warrington, R., Watson, W., et al., An introduction to immunology and immunopathology, *Allergy, Asthma, and Clinical Immunology*, Vol. 14 (Supplement 2) (2018), art. 49.

- They are **antigen-independent** (non-specific), i.e. they do not adapt specifically to the antigen but rather destroy all pathogens with certain molecular properties.
- has **no immunologic memory** and, therefore, it is unable to recognize or “memorize” the same pathogen should the body be exposed to it in the future.
- **Main actors are macrophages**, which are effector cells secreted by phagocytose bacteria. They themselves secrete both pro-inflammatory and antimicrobial mediators. In addition, macrophages play an important role in eliminating diseased and damaged cells through their programmed cell death.

Adaptive immunity:

- The process is started quickly but lasts longer in time.
- Is **antigen-dependent and antigen-specific**, thereby requiring lag time between exposure to the antigen and maximal response to produce the right molecules and cells to attack the pathogen.
- Has the **capacity for memory** which enables the host to mount a more rapid and efficient immune response upon subsequent exposure to the antigen.
- **Main actors are** immune cells such as **T cells, B cells, natural killer cells (NKCs), and antibodies** such as immunoglobulin A.

3. Mechanisms of action of ingredients

2.1 Immunell™

2.1.1 Description

Immunell™ is a unique immune promoting ingredient:

- **a nucleotide-based immune-modulator**, i.e. a useful tool to strengthen and maintain the balance of a healthy immune system, keeping it ready for when resistance support is needed.
- Natural compounds **extracted from yeast** (*Saccharomyces cerevisiae*)
- **a vegan-appropriate alternative to** nucleotide supplementation by dairy cow **colostrum**.
- Has **very high absorption and bioavailability** as small molecular size (10,000 kilodaltons) of the nucleotides makes them capable of transferring a cellular immune response and optimizing immune system response and balance.
- **a unique source of the 4 semi-essential nucleotides** Adenine, Cytosine, Guanine and Uracil (60% of the content) that act as nutrients necessary for increased cell division in the gastrointestinal and immune systems.
- **High and stable purity rate** (standardized to 60% nucleotides).
- Nucleotides **trigger rapid immune response**. Clinically proven to induce rapid changes in immune function **within 30 minutes to 2 hours**.
- Naturally occurring in food and breast milk and found in all living cells of the body, nucleotides are semi-essential molecules. Nucleotides have been used for years in nutrition to support organ growth, immune system and intestinal function.

2.1.2 Nucleotide supplementation activates the immune system on several levels

Nucleotides have been used for years in nutrition to support organ growth, immune system and intestinal function. Stressful lifestyle, strenuous workout, etc.) or certain organs (fast renewal tissues like the intestines) can lead to a high need for nucleotides.

Nucleotide supplementation has been studied for several decades:

- In vitro, it induces an array of immunoactive molecules essential to immune system communication.
- The direct effects of nucleotides on separate aspects of our innate immune defense were tested. The results showed:
 - **Activation of phagocytes and increase in the intensity of phagocytosis**
 - Tested on human polymorphonuclear (PMN) cells.
 - The action was almost immediate.
 - Within a few minutes, treated phagocytic cells consumed more particles and more cells were activated as phagocytes.
 - Phagocytes produce macrophages that are part of the innate immune system.
 - **Activation of Natural Killer cells (NKCs).**
 - NKCs are able to respond immediately to invading pathogens and attack cells that were invaded by viruses, or other pathogens.
 - Part of the adaptive immune system response.
 - Treatment of NK cells with Immunell resulted in an activation of the NK cells. The treated NK cells expressed much higher amounts of an activation marker called CD69, which indicates that the NK cells were activated to be more efficient at attacking target cells.

Expression of the NK cell activation marker CD69

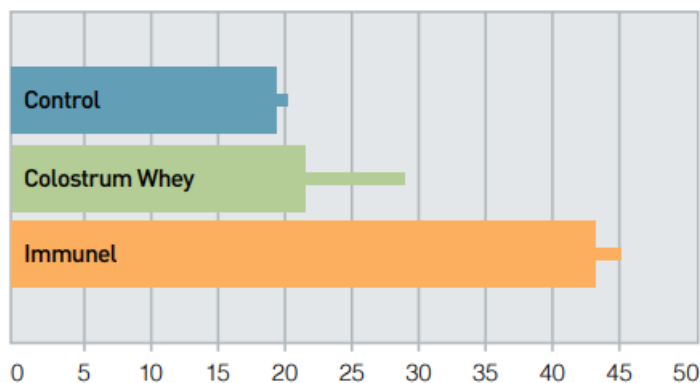


Fig. 2 Percent Positive NK Cells

2.2 Zinc

Zinc has a double role, as modulator of thymulin, a compound essential for the life of T cells, and as activator of antioxidant processes.

2.2.1 Zinc is essential for the life of T cells

T lymphocytes, white blood cells (leukocytes) are crucial components of the natural immune system.

T cells are the basis for the adaptive immune system because they determine the specificity of immune response to antigens (foreign substances) in the body. Their contact with the antigen results in interleukin 2 (IL-2) production, proliferation, and differentiation to effector cells that migrate to diverse sites to promote pathogen clearance.⁷

T cells coordinate multiple aspects of adaptive immunity throughout life and can mirror-copy the antigens (foreign substances) in the body, attack and destroy them, or instruct other responses from the body.

Thymulin binds to high-affinity receptors on T cells, induces several T-cell markers, and promotes T-cell function, including allogenic cytotoxicity, suppressor functions, and interleukin-2 (IL-2) production.

Zinc is required for biological activity of thymulin, which is essential for T cell life.⁸

2.2.2 Zinc is essential in antioxidative relief processes

Modulator of oxidative stress processes

Zinc also plays a role in modulating oxidative stress by inhibiting the NADPH oxidases, enzymes that produce oxidative stress, by inducing the production of metallothionein, which is very rich in cysteine, and an excellent scavenger of free radicals.

Reducer of inflammatory cytokines

As a result of zinc deficiency, the macrophages-monocytes are stressed and they generate inflammatory cytokines such as TNF- α and IL-1 β , which are known to generate free radicals. Zinc negatively regulates gene expression of inflammatory cytokines such as TNF- α and IL-1 β .⁹

2.3 Vitamin C

Vitamin C role in the immune system is multifaceted but well documented.¹⁰

Vitamin C:

- **Supports epithelial (gut) barrier function** against pathogens.
- **Promotes the oxidant scavenging activity**, thereby protecting against oxidative stress.
- **Accumulates in phagocytic cells**, such as neutrophils, and **can enhance chemotaxis, phagocytosis**, generation of reactive oxygen species, and ultimately microbial killing.
- **Needed for apoptosis** (programmed cell death) and clearance of the spent neutrophils from sites of infection by macrophages, thereby decreasing necrosis/NETosis and potential tissue damage.
- The role of vitamin C in lymphocytes is less clear, but it has been **shown to enhance differentiation and proliferation of B- and T-cells**, likely due to its gene regulating effects.

⁷ Kumar, B.V., Connors, T. J., Farber, D. L., Human T Cell Development, Localization, and Function throughout Life, Immunity, Vol. 48, Issue 2 (2018), pp. 202-213.

⁸ Beck, F. W. J., et al. Changes in cytokine production and T cell subpopulations in experimentally induced zinc-deficient humans, American Journal of Physiology, Vol. 272 (1997), pp. E1002–E1007.

⁹ Prasad, A. S., et al., Anti-oxidant effect of zinc in humans, Free Radical Biology and Medicine, Vol. 37 (2004), pp. 1182–1190.

¹⁰ Carr, A. C., Maggini, S., Vitamin C and Immune Function, Nutrients, Vol. 9, Issue 11 (2017), art. 1211.

- **Vitamin C deficiency results in impaired immunity** and higher susceptibility to infectio

