# INTEGRATION OF INNOVATIONS IN REGENERATIVE MEDICINE: THE JOINT APPROACH OF THE MEDICAL CENTER - STEM CELL BANK "PHOENIX" AND JSC FROCETH

Leading global research and medical services in the field of regenerative medicine, anti-aging, autoimmune, oncological, and degenerative diseases.




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# WHO ARE WE

## **FROCETH:** Pioneers in Biotechnology in Lithuania and Europe

FROCETH is the first and only biotechnological company in Lithuania, specialized in the production of personalized medicinal products for modern therapy. Established in 2014, the company combines the experience and knowledge of leading specialists in the fields of biomedical sciences, molecular biology, and biotechnology. We have established a unique tissue bank and clinic, which are the first of their kind in Lithuania and the European Union, operating in accordance with the standards of Good Manufacturing Practice (GMP) and Advanced Therapy Medicinal Products (ATMP) under European Regulation No. 726/2004 and Directive No. 2001/83/EC. Treatment is carried out under European legislation for "HOSPITAL EXEMPTION CLAUSE IN ADVANCED THERAPY MEDICAL PRODUCTS." Our mission is to invest in scientific research and the development of new products to meet the individual needs of each patient. Patent for development

## **Phoenix Medical Center:** Leaders in Regenerative Medicine

Located in Plovdiv, Bulgaria, the Phoenix Medical Center is a leading clinic in the field of stem cell treatment and regenerative medicine. Our mission is to provide personalized and innovative treatment that meets the specific needs of each of our patients. Thanks to the years of experience and expertise of our medical staff and scientific researchers, we are engaged in various scientific projects and clinical trials. This allows us to be at the forefront of scientific progress and to offer our patients the most advanced therapeutic options.

## **Innovative Healthcare:** Collaborative Efforts of FROCETH and Phoenix Medical Center

By uniting the efforts and professionalism of FROCETH and Phoenix Medical Center, we strive to provide leading treatment in the field of biotechnology and regenerative medicine. Our common goal is to create innovative therapies and improve the quality of life of our patients by offering personalized medical solutions that meet the highest standards of quality.

# PRODUCTS AND DEVELOPMENTS IN OUR LABORATORIES

Our company FROCETH, as the only biotechnological company in Lithuania and one of the few in the European Union, holds a license for the production of Investigational Medicinal Products for Modern Therapy (TPTVP) with number 0914.-ATMP.

In collaboration with Phoenix Medical Center, we are actively working on expanding our research and production capabilities, focusing on the development and application of stem cell and exosome transplantation intramedullary and intracavernously.

These methods represent innovative approaches in the treatment and restoration of damaged tissues and organs, which is a fundamental component of regenerative medicine.

They are innovative approaches in the treatment of various diseases, including diabetes, erectile dysfunction, multiple sclerosis, Alzheimer's, liver diseases, cancer, autoimmune diseases, degenerative diseases, and many other ailments.

We pay special attention to anti-aging therapies, which are of significant importance for improving the quality of life and slowing down the aging processes.

Additionally, our company focuses on developing an innovative TCV vaccine, which represents an important step in the field of immunotherapy and the prevention of autoimmune diseases. This development is part of our efforts to offer new therapeutic options and to expand the boundaries of contemporary medical science.

Through our joint work with Phoenix Medical Center, we reach patients from various parts of the world: Europe, Asia, America.



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# Our Developments

- Our Patents
- **Anti-tumor Leukocyte Therapy -** contains leukocytes designed to inhibit tumor development and can be effective against various types of cancer.
- **Antibacterial Leukocyte Therapy -** composed of leukocytes with high antibacterial activity.
- **Antiviral Leukocyte Therapy -** composed of leukocytes with high antiviral activity.
- **Tissue Protective Leukocyte Therapy** – consists of leukocytes intended to contribute to tissue regeneration processes and is suitable for treating joint and skin diseases.
- **Platelet (PRP) Therapy –** consists of platelets and is suitable for treating joint and skin diseases.
- **Serum (Orthokine) Therapy -** contains anti-inflammatory and growth factors secreted by cells during the blood clotting process. It is intended for the treatment of joint and skin diseases.
- **T-cell Therapy-** includes vaccine T-cells capable of inducing anti-idiotypic immune responses, targeted against autoimmune or allergic T-lymphocytes.
- **Cellular Anti-Tumor Vaccine -** contains immune cells effective in initiating tumor-specific immune responses.

# STEM CELLS

In our laboratories, using patented technology, we process and isolate stem cells from umbilical cord, with a focus on the so-called „Muse stem Cells“

- Muse stem Cells (Multi-lineage differentiating stress enduring cells)

  Patent MUSE STEM CELLS

Muse stem Cells (Multi-lineage differentiating stress enduring cells) are endogenous, non-cancerous, pluripotent stem cells. These cells are found in the tissues of almost every organ, including the umbilical cord, bone marrow, and peripheral blood. Muse Cells do not belong to the already studied types of stem cells, making them unique in their class.

The intriguing aspect of Muse Cells is their ability to generate cells representing all three germ layers from a single cell, both spontaneously and under the influence of cytokines. They express genes for pluripotency and are characterized by their capability for triploblastic differentiation, which is renewed over generations.

It is particularly important that when transplanted into a living organism, **Muse Cells do not cause the formation of teratomas.** This is largely due to their low telomerase activity, which reduces the risk of tumorigenesis through uncontrolled cell proliferation..

Muse Cells have been identified as cells positive for SSEA-3+, a known marker for undifferentiated human embryonic stem cells. This makes them easily recognizable in laboratory conditions and opens the door to their numerous applications. In addition, they are also positive for common markers of mesenchymal stem cells such as CD105, CD90, CD29, and CD73, which makes them uniquely double positive for both pluripotent and mesenchymal stem cells.



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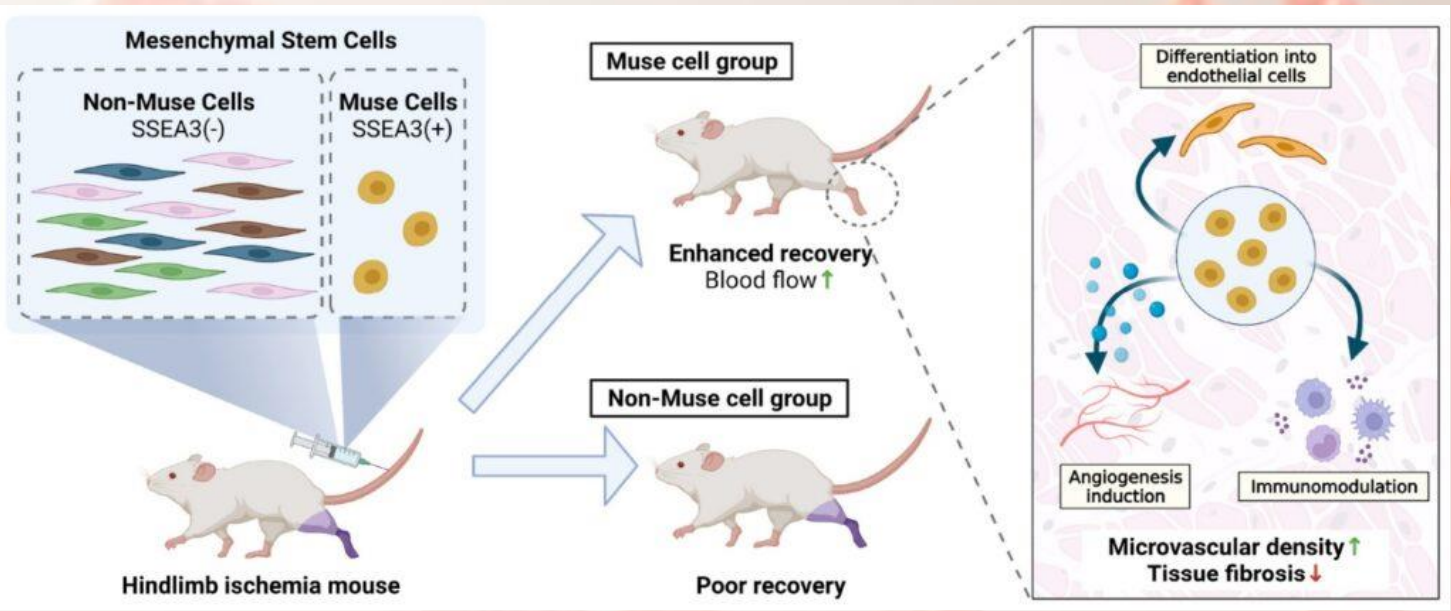
COMPARISON OF STEM CELL SOURCES

| Type of Stem Cells | Differentiation Potential | STRESS RESISTANCE/ RISK OF REJECTION | Source | Clinical Applications |
|---|---|---|---|---|
| **Multi-lineage differentiating stress enduring cells (MUSE CELLS)** | **Multiline** | **High / Low** | **Specialized Cultivation Conditions** | **Regenerative Medicine, research on diseases such as diabetes, erectile dysfunction, multiple sclerosis, anti-aging, Alzheimer's, liver diseases, cancer, autoimmune diseases.** |
| Embryonic Stem Cells | Pluripotent | Low / High | Blastocysts / Embryos | Research, Potential Therapies. |
| Adult Stem Cells (ASCs): | Multipotent | Moderate / Low | Different adult tissues | Transplants, treatment of diseases |
| Induced Pluripotent Stem Cells" (iPSCs) | Pluripotent. | Low / Moderate to high | Genetically Reprogrammed Adult Cells | Regenerative Medicine, Disease Modeling. |

Table 1. Visual Comparison between Ordinary Stem Cells and Muse Stem Cells. Positive for SSEA3(+).



## Characteristics of Muse Cells and Their Significance

- Stress Resilience

Muse Cells exhibit exceptional stress tolerance. This ability makes them suitable for use in environments that may be harmful to other cell types, providing them an advantage in regenerative medicine.

- Lack of Tumorigenicity

Muse Cells do not show a tendency to form tumors. This makes them a safe choice for clinical applications, reducing the risk of unwanted cell proliferation.

- Genotoxic Stress Resistance

Thanks to their efficient detection of DNA damage and activation of DNA repair systems, Muse Cells are resistant to genotoxic stresses. This makes them particularly valuable in environments where DNA may be damaged.

- Isolation as SSEA-3 Positive Cells

Muse Cells can be isolated as SSEA-3 positive cells, a known marker for human embryonic stem cells. This marker ensures that the cells are "fresh" and suitable for research and therapeutic applications.

- Pluripotency and Self-Renewal Capacity

As pluripotent stem cells, Muse Cells can generate various cell types representing all three germ layers. This self-renewal capacity is especially important for long-term therapeutic processes.

- Non-Tumorigenic with Low Telomerase Activity

The low telomerase activity in Muse Cells makes them non-tumorigenic, which is crucial for the safety of stem cell-based therapies.

- Selective Accumulation in Damaged Tissues



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Muse Cells selectively accumulate in damaged tissues through intravenous or local injections, using the S1P-S1P receptor 2 axis. This facilitates their targeting to specific damaged areas in the body.

- Restoration of Functional Cells

After entering the damaged tissues, Muse Cells spontaneously differentiate into tissue-compatible cells, which is key for effective tissue restoration.

- Tissue Restoration via Systemic Application

Muse Cells can be used for tissue restoration through systemic application, facilitating their use in various medical procedures.

- Represent a small percentage of bone marrow and mesenchymal stem cell transplants

Muse Cells make up about 0.03% of bone marrow transplants and a few percent of mesenchymal stem cell transplants, highlighting their specificity and value.

- Have immunosuppressive and immunomodulatory effects

These cells play a crucial role in modulating the immune system, which can be of vital importance in the treatment of autoimmune diseases and in transplantations.

- Directly obtained from normal human tissues

Pluripotent stem cells can be directly obtained from normal human tissues, eliminating the need for artificial manipulations such as gene introduction.

- Direct use of donor Muse Cells for treatment

Donor Muse Cells can be directly used for treatment without the need for HLA compatibility tests or immunosuppressive treatments, thanks to their specific immune privilege.

## The 'Magical' Markers of Muse Cells

Muse Cells have been identified as SSEA-3+ positive cells, a well-known marker for undifferentiated human embryonic stem cells. This makes them easily recognizable in laboratory conditions and opens the door to their numerous applications. Additionally, they are positive for common markers of mesenchymal stem cells such as CD105, CD90, CD29, and CD73, making them uniquely double-positive for both pluripotent and mesenchymal stem cells.

## Possibilities for Differentiation of Muse Cells

Muse Cells are impressive with their ability to transform into various types of cells, which is crucial for their use in regenerative medicine. Let's explore their differentiation potential in a convenient and easy-to-understand manner.

## In Laboratory Conditions (In vitro)

Muse Cells can differentiate into cells from the following three primary cell layers:

1. **Ectodermal Cells:** This includes cells that develop into the skin and nervous system, such as:

Neuronal markers like nestin, NeuroD, Musashi, neurofilament, MAP-2.

Melanocytic markers like tyrosine's, MITF, gf100, TRP-1, DCT.

2. **Mesodermal Cells**: These cells develop into structures like bones, muscles, and blood, including:

Markers such as brachyury, Nkx2-5, smooth muscle actin.

Osteocalcin, oil red-positive fat droplets, desmin.

3. **Endodermal Cells:** They develop into internal organs such as the liver and pancreas, with markers like:

GATA-6, alpha-fetoprotein, cytokeratin-7, albumin.



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These abilities make Muse Cells highly versatile and promising for various regenerative medicine applications.

## In a Living Organism (In vivo)

In natural conditions, Muse Cells are directed towards the sites of injury in the body and spontaneously differentiate into cells compatible with the damaged tissue. This has been demonstrated in numerous studies where human Muse Cells have been introduced into animal models with various diseases and injuries, including:

- Acute liver disease (fulminant hepatitis).
- Partial hepatectomy.
- Muscle degeneration.
- Epidermolysis bullosa (skin disease).
- Skin injuries.
- Stroke.
- Spinal cord injury.
- Autoimmune diseases.
- Joint diseases.

The ability of Muse Cells to differentiate into various cell types and participate in tissue regeneration makes them exceptionally important in the field of regenerative medicine. They offer a promising avenue for the recovery and treatment of various types of injuries and diseases.

## Safety of Muse Cells: Lack of Tumorigenicity

Muse Cells are distinguished by a very important characteristic - they are safe and do not trigger tumor formation.

# WHARTON'S JELLY MESENCHYMAL STEM CELLS EXOSOMES

## What do exosomes represent

Exosomes are extracellular vesicles generated by cells carrying nucleic acids, proteins, lipids, and metabolites. They serve as mediators of both local and distant intercellular communication in health and disease, affecting various aspects of cellular biology.

Studying extracellular vesicles (EVs) has the potential to identify unknown cellular and molecular mechanisms in intercellular communication and organ homeostasis during disease. Exosomes, with an average diameter of approximately 100 nanometers, are a subgroup of EVs. The biogenesis of exosomes involves their origin in endosomes and subsequent interactions with other intracellular vesicles and organelles, which determine the final content of exosomes. Their diverse constituents include nucleic acids, proteins, lipids, amino acids, and metabolites, which can reflect their cellular origin.

In various diseases, exosomes provide insights into altered cellular or tissue states, and their detection in biological fluids potentially offers multi-component diagnostic insights. The efficient exchange of cellular components through exosomes can inform their potential utility in designing exosome-based therapies.

Exosomes are associated with immune responses, viral pathogenesis, cardiovascular diseases, and central nervous system-related disorders. Proteins, metabolites, and nucleic acids delivered by exosomes to recipient cells effectively alter their biological



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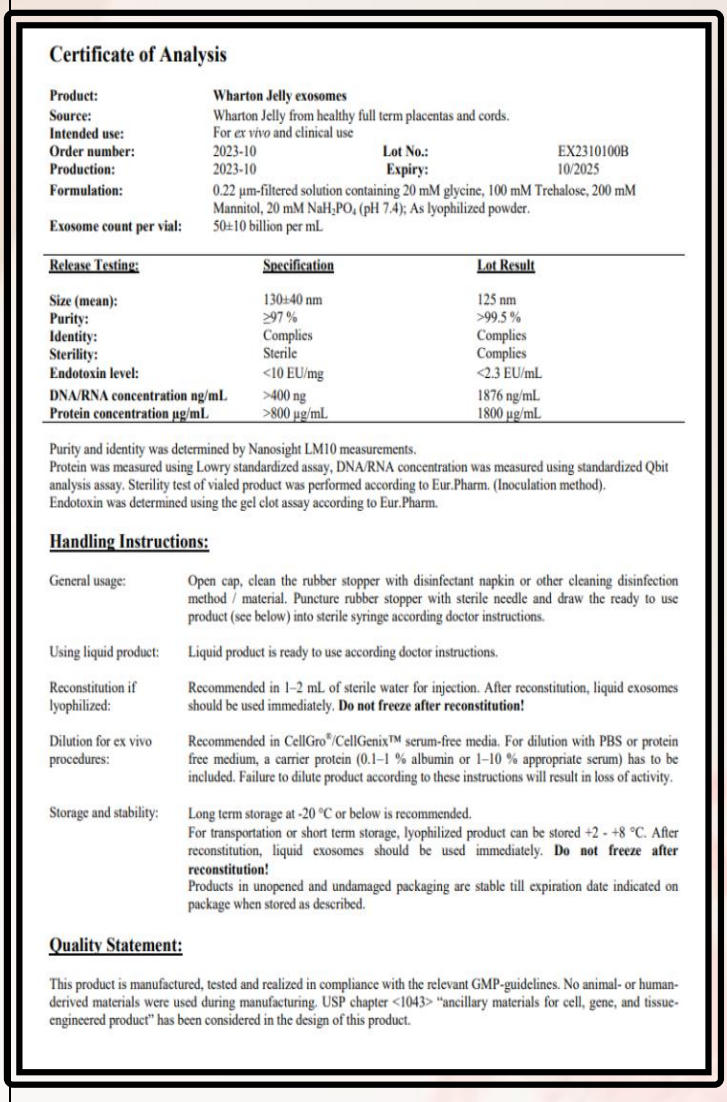
response Such exosome-mediated reactions can limit the progression of diseases. The intrinsic properties of exosomes in regulating complex intracellular pathways have increased their potential utility in the therapeutic management of various conditions, including neurodegenerative diseases and cancer. Exosomes can be engineered to deliver various therapeutic cargoes, including short interfering RNA, antisense oligonucleotides, chemotherapeutic agents, and immunomodulatory, with the potential for targeted delivery to desired sites.

# ANALYSES AND CERTIFICATES



**Certificate of Analysis**

| | | | |
|---|---|---|---|
| **Product:** | **Wharton Jelly exosomes** | | |
| **Source:** | Wharton Jelly from healthy full term placentas and cords. | | |
| **Intended use:** | For *ex vivo* and clinical use | | |
| **Order number:** | 2023-10 | **Lot No.:** | EX2310100B |
| **Production:** | 2023-10 | **Expiry:** | 10/2025 |
| **Formulation:** | 0.22 µm-filtered solution containing 20 mM glycine, 100 mM Trehalose, 200 mM Mannitol, 20 mM $NaH_2PO_4$ (pH 7.4); As lyophilized powder. | | |
| **Exosome count per vial:** | 50±10 billion per mL | | |

| Release Testing: | Specification | Lot Result |
|---|---|---|
| **Size (mean):** | 130±40 nm | 125 nm |
| **Purity:** | ≥97 % | >99.5 % |
| **Identity:** | Complies | Complies |
| **Sterility:** | Sterile | Complies |
| **Endotoxin level:** | <10 EU/mg | <2.3 EU/mL |
| **DNA/RNA concentration ng/mL** | >400 ng | 1876 ng/mL |
| **Protein concentration µg/mL** | >800 µg/mL | 1800 µg/mL |

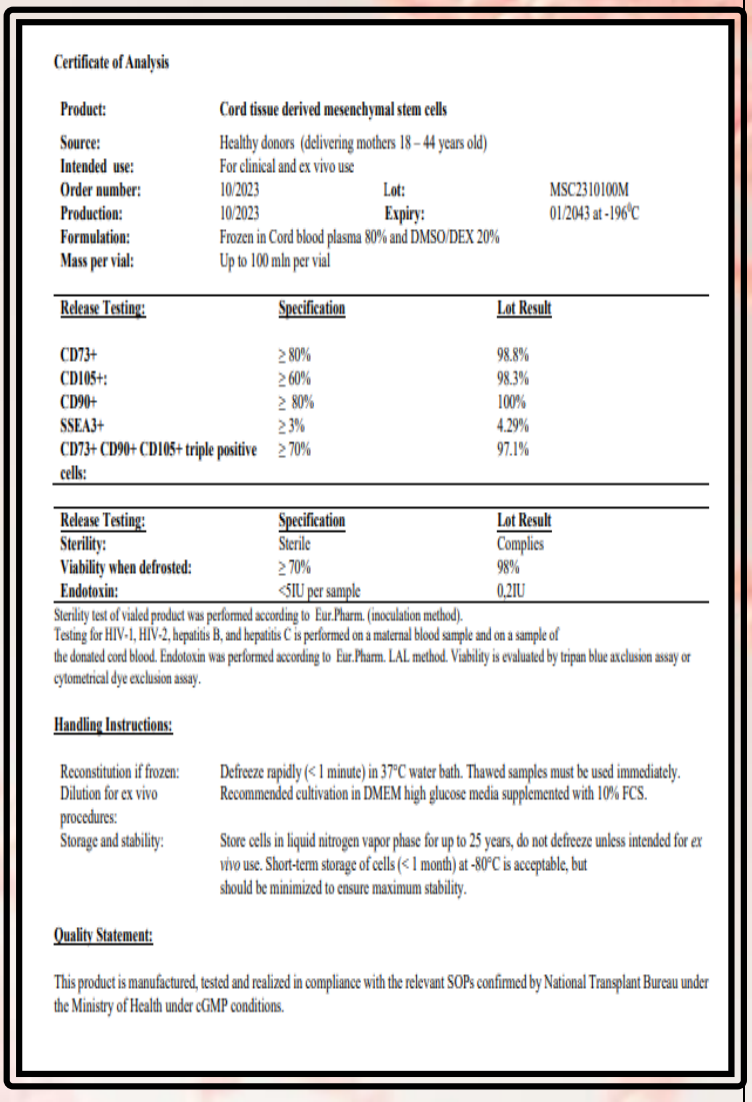
Purity and identity was determined by Nanosight LM10 measurements.
Protein was measured using Lowry standardized assay, DNA/RNA concentration was measured using standardized Qbit analysis assay. Sterility test of vialed product was performed according to Eur.Pharm. (Inoculation method).
Endotoxin was determined using the gel clot assay according to Eur.Pharm.

**Handling Instructions:**

| | |
|---|---|
| General usage: | Open cap, clean the rubber stopper with disinfectant napkin or other cleaning disinfection method / material. Puncture rubber stopper with sterile needle and draw the ready to use product (see below) into sterile syringe according doctor instructions. |
| Using liquid product: | Liquid product is ready to use according doctor instructions. |
| Reconstitution if lyophilized: | Recommended in 1–2 mL of sterile water for injection. After reconstitution, liquid exosomes should be used immediately. **Do not freeze after reconstitution!** |
| Dilution for ex vivo procedures: | Recommended in CellGro®/CellGenix™ serum-free media. For dilution with PBS or protein free medium, a carrier protein (0.1–1 % albumin or 1–10 % appropriate serum) has to be included. Failure to dilute product according to these instructions will result in loss of activity. |
| Storage and stability: | Long term storage at -20 °C or below is recommended. For transportation or short term storage, lyophilized product can be stored +2 - +8 °C. After reconstitution, liquid exosomes should be used immediately. **Do not freeze after reconstitution!** Products in unopened and undamaged packaging are stable till expiration date indicated on package when stored as described. |

**Quality Statement:**

This product is manufactured, tested and realized in compliance with the relevant GMP-guidelines. No animal- or human-derived materials were used during manufacturing. USP chapter <1043> "ancillary materials for cell, gene, and tissue-engineered product" has been considered in the design of this product.



**Certificate of Analysis**

| | | | |
|---|---|---|---|
| **Product:** | **Cord tissue derived mesenchymal stem cells** | | |
| **Source:** | Healthy donors (delivering mothers 18 – 44 years old) | | |
| **Intended use:** | For clinical and ex vivo use | | |
| **Order number:** | 10/2023 | **Lot:** | MSC2310100M |
| **Production:** | 10/2023 | **Expiry:** | 01/2043 at -196°C |
| **Formulation:** | Frozen in Cord blood plasma 80% and DMSO/DEX 20% | | |
| **Mass per vial:** | Up to 100 mln per vial | | |

| Release Testing: | Specification | Lot Result |
|---|---|---|
| **CD73+** | ≥ 80% | 98.8% |
| **CD105+:** | ≥ 60% | 98.3% |
| **CD90+** | ≥ 80% | 100% |
| **SSEA3+** | ≥ 3% | 4.29% |
| **CD73+ CD90+ CD105+ triple positive cells:** | ≥ 70% | 97.1% |

| Release Testing: | Specification | Lot Result |
|---|---|---|
| **Sterility:** | Sterile | Complies |
| **Viability when defrosted:** | ≥ 70% | 98% |
| **Endotoxin:** | <5IU per sample | 0,2IU |

Sterility test of vialed product was performed according to Eur.Pharm. (inoculation method).
Testing for HIV-1, HIV-2, hepatitis B, and hepatitis C is performed on a maternal blood sample and on a sample of the donated cord blood. Endotoxin was performed according to Eur.Pharm. LAL method. Viability is evaluated by tripan blue axclusion assay or cytometrical dye exclusion assay.

**Handling Instructions:**

| | |
|---|---|
| Reconstitution if frozen: | Defreeze rapidly (< 1 minute) in 37°C water bath. Thawed samples must be used immediately. |
| Dilution for ex vivo procedures: | Recommended cultivation in DMEM high glucose media supplemented with 10% FCS. |
| Storage and stability: | Store cells in liquid nitrogen vapor phase for up to 25 years, do not defreeze unless intended for *ex vivo* use. Short-term storage of cells (< 1 month) at -80°C is acceptable, but should be minimized to ensure maximum stability. |

**Quality Statement:**

This product is manufactured, tested and realized in compliance with the relevant SOPs confirmed by National Transplant Bureau under the Ministry of Health under cGMP conditions.

# THERAPEUTIC PRACTICES AND PACKAGES

We have created this special treatment plan based on your condition!

## The treatment includes:

- Blood tests
- Ultrasonography
- Detoxification / blood apheresis /
- Intravenous infusions with: water-soluble and fat-soluble vitamins, amino acids, phospholipids, NAD+, Exosomes
- Intravenous infusions of stem cells and exosomes, local application of stem cells and exosomes according to the patient's condition.
- Treatment, hormonal stimulation, nerve damage recovery, Fisetin IM + dry exosome injections to reduce the levels of so-called "Zombie cells"
- Venous and muscular application of placental extracts
- Restoration of tissue homeostasis.

The quantity of intravenously administered stem cells and intravenous and locally administered exosomes can vary between 100-200 million for stem cells and 1-2 trillion for exosomes at the beginning of the treatment course.

**Treatment duration: 7-14 days.**

**Date: January 24. 2024.**



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