

WORKFLOW SOLUTIONS

Blood Fractionation & Analysis

MP BIOMEDICALS

www.mpbio.com

From Blood to Drug Discovery & Next Generation Treatments

Blood and blood-derived samples have played a critical role in the field of drug discovery and next generation treatments. The ability to isolate and analyze specific components of blood, such as DNA, proteins, red blood cells, and white blood cells, has become an essential aspect of pre-clinical and clinical studies. As a result, it is crucial to utilize high-quality tools and reagents to ensure proper separation of blood components and expansion of healthy primary cells during research.

MP Biomedicals offers a diverse range of premium products to support blood-derived research. Our ready-to-use LSM™ and M-PRM™ solutions enable proper gradient separation of plasma, red blood cells, and white blood cells for analysis of specific blood components. We also supply a comprehensive selection of media, supplements, reagents, and kits to support your downstream workflows, from cell maintenance in a serum or animal-free environment to cell-based assays and DNA, RNA, and protein analysis.

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CHAPTER 1

Introduction

While blood is often referred to as a “liquid organ”, it is not technically an organ, but rather a bodily fluid that plays a crucial role in maintaining the human body’s proper function. Blood is made up of several different components, including red blood cells, white blood cells, platelets, and plasma, and each has a specific function.

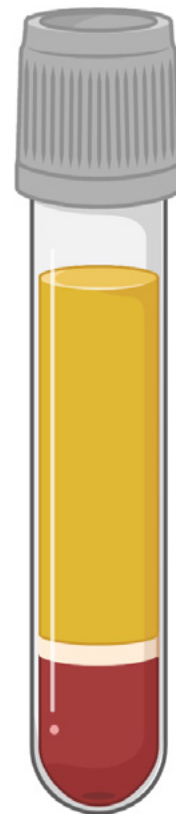
Red blood cells (RBCs): RBCs contain hemoglobin, a protein that binds to oxygen and carries it from the lungs to the body’s tissues. RBCs are essential for delivering oxygen to the body and removing carbon dioxide from tissues.

White blood cells (WBCs): WBCs are responsible for fighting infections and other diseases. They help the body identify and destroy bacteria, viruses, and other harmful substances that can cause illness.

Platelets: Platelets are essential for blood clotting, which is the process by which the body stops bleeding after an injury. When a blood vessel is damaged, platelets clump together to form a plug that slows bleeding.

Plasma: Plasma is the liquid component of blood that carries the blood cells and other proteins throughout the body. It also contains important substances, such as electrolytes and hormones.

Each of these blood components is essential for maintaining the health and well-being of the human body. When any of these components are missing or not functioning properly, it can lead to serious health problems and even death.



White Blood Cells

White blood cells, also known as leukocytes, play a crucial role in the body's immune system. They are responsible for protecting the body against various pathogens, including viruses, bacteria, and other harmful microorganisms. White blood cells are derived from blood, and their isolation and analysis have become an essential aspect of drug discovery and next-generation treatments.

In drug discovery, white blood cells play a vital role in the pre-clinical stage. They are used to screen and identify potential drug candidates that can selectively target specific immune cells or modulate immune response pathways. By isolating and analyzing white blood cells, researchers can gain a better understanding of the immune system's complex mechanisms and develop drugs that can target specific immune cell subsets or modify their functions.

Furthermore, white blood cells are used in next-generation treatments, such as immunotherapy. Immunotherapy is a type of cancer treatment that uses the patient's immune system to target and destroy cancer cells. In some cases, white blood cells can be isolated from the patient's blood, modified or engineered ex-vivo, and reintroduced into the patient to enhance the immune system's response against cancer cells. This type of therapy has shown promising results in treating various types of cancer, including leukemia, lymphoma, and solid tumors.

The isolation and analysis of white blood cells has also paved the way for personalized medicine. With advancements in technologies such as single cell sequencing and mass cytometry, researchers can now analyze individual white blood cells and gain a more comprehensive understanding of the patient's immune system. This information can be used to tailor treatments specific to the patient's immune profile and improve treatment outcomes.

To obtain white blood cells, or more specifically, peripheral blood mononuclear cells (PBMCs), blood is first collected from a donor and then mixed with an anticoagulant to prevent clotting. The blood sample is then subjected to density gradient centrifugation, where it is separated based on the difference in the density of the various components. This process can be performed using a density gradient medium to isolate the PBMCs, which form a distinct layer between the plasma and the red blood cells. The isolated PBMCs can be washed and resuspended in a culture medium for further expansion or analysis.



CHAPTER 2

One-Step Whole Blood Fractionation

PLASMA, PBMCS, LYMPHOCYTES, MONOCYTES, GRANULOCYTES, AND ERYTHROCYTES

MP Bio offers ready-to-use **Lymphocyte Separation Medium (LSM™)** and **Mono-Poly Resolving Medium (M-PRM™)**, which provide an efficient one-step blood fractionation solution. This allows for valuable insights into fundamental biology and supports the development of new plasma or immune derived therapies. The solutions facilitate the one-step separation of plasma, PBMCs, lymphocytes (B cells & T cells), monocytes, granulocytes, and erythrocytes from whole blood, making it easier for researchers to isolate and study specific blood components from a single sample.

Analyzing plasma biomarkers pre- and post-treatment can provide insight for cancer diagnosis and treatment, help study the mechanisms of cancer progression, and aid in the development of targeted therapies to improve patient outcomes. Furthermore, lymphocytes, monocytes, and granulocytes are all vital components of the body's immune system and play significant roles in fighting against cancer. B and T cells can directly attack cancer cells, while macrophages and neutrophils can engulf and digest them. Understanding the complex interactions between these immune cells and cancer cells can lead to the development of more effective treatments that harness the power of the immune system to fight cancer.



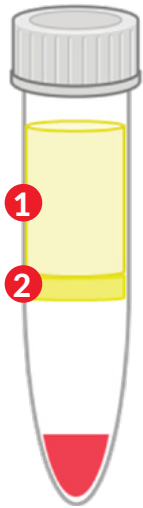
Lymphocyte Separation Medium

Density: 1.077 g/mL



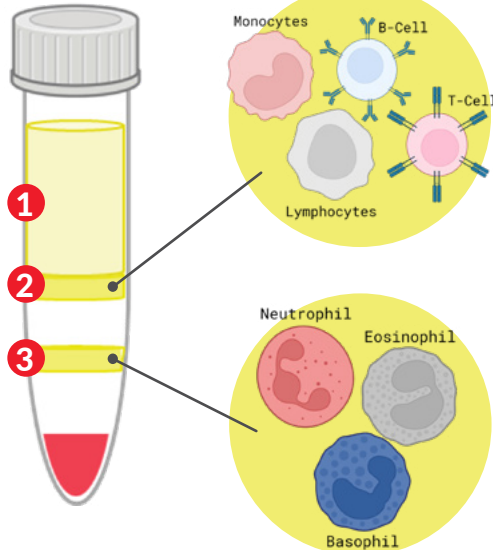
Mono-Poly Resolving Medium

Density: 1.114 g/mL



Lymphocyte Separation Medium

Density: 1.077 g/mL



Mono-Poly Resolving Medium

Density: 1.114 g/mL

1 Plasma Layer

- Platelets (2–4 μm)
- Antibodies
- Cytokines
- Hormones
- Electrolytes

2 Lymphocytes & Monocytes Layer

- T cells
- B cells
- NK cells

3 Granulocytes Layer

- Neutrophils
- Basophils
- Eosinophils

Learn more about our products for one-step whole blood fractionation:

Name	Cat. No.
LSM™ Lymphocyte Separation Medium	0850494
LymphoSep™ Lymphocyte Separation Medium	0916922
LymphoSpinner™ Lymphocyte Isolation Tube, 15 mL	091692425
LymphoSpinner™ Lymphocyte Isolation Tube, 50 mL	091692525
LymphoSpinner™ Sample Ready Lymphocyte Isolation Tube, 15 mL	091692625
LymphoSpinner™ Sample Ready Lymphocyte Isolation Tube, 50 mL	091692725
Mono-Poly™ Resolving Medium	091698049



CHAPTER 3

Maintenance, Expansion, & Treatment

Peripheral blood mononuclear cells (PBMCs) are a collection of white blood cells that play a crucial role in the body's immune response. They are easily accessible through a simple blood draw and a valuable resource for drug discovery. PBMCs are composed of several different types of immune cells, including:

- T cells
- B cells
- Natural killer cells
- Monocytes
- Dendritic cells

PBMCs play a role in the immune system's ability to fight disease. Because of their attainability and versatility, PBMCs are widely used in drug discovery to identify and develop new therapies for a variety of diseases, including cancer, autoimmune disorders, and infectious diseases.

The choice of basal media for culturing peripheral blood mononuclear cells (PBMCs) in vitro depends on the experimental requirements and the specific cell types being cultured.

RPMI 1640 is a commonly used medium for PBMC culture, as it supports the growth of lymphocytes, monocytes, and other immune cells. It contains a balanced mixture of amino acids, vitamins, minerals, and other nutrients that support cell growth and proliferation.

DMEM is another commonly used medium that can be used to culture PBMCs. It contains a high glucose concentration and other nutrients that support cell growth, proliferation, and survival. DMEM can also be supplemented with serum to provide additional growth factors and nutrients.

MP Bio takes pride in our extensive portfolio of media products to support diverse experimental designs. Boost your cell culture success with MP Bio's media components for robust immune cell growth and functionality.

Recommended Components for Immune Cell Culture Medium



Media/Component	T Cells	B Cells	Monocytes	NK Cells	Neutrophils	Basophils	Eosinophils
Basal Media	DMEM	•			•	•	
	RPMI-1640	•	•	•	•	•	•
	IMDM	•	•		•	•	
Proteins	IL-2	•	•	•	•		
	IL-3					•	•
	IL-4		•	•		•	
	IL-5						•
	IL-10		•				
	IL-15				•		
	IL-21				•		
	GM-CSF			•			
	G-CSF					•	
SCF						•	•
Amino Acids	Glutamine	•	•	•	•	•	
	SupraGLN™	•	•	•	•	•	
Serum	FBS	•	•	•	•	•	•
	Human Serum	•	•		•		
	FastGro™	•	•	•	•	•	•

FastGro™, Fully Chemically Defined FBS Replacement

Fetal bovine serum (FBS) is widely used as a serum-supplement for in vitro cell culture media. FBS promotes healthy cell growth by providing an undefined mixture of nutrients, such as proteins, attachment factors, growth factors, lipids and hormones. However, due to its undefined nature, FBS can lead to unexpected and undesired stimulation of cells. There is also biorisk from animal protein or pathogen contamination, including risk of bovine spongiform encephalopathy (BSE).

To avoid these concerns, MP Bio has launched **FastGro™**, a fully chemically defined FBS replacement for cell culture use. It allows culturing a wide range of cells in vitro without the use of serum or any animal or human derived compound.

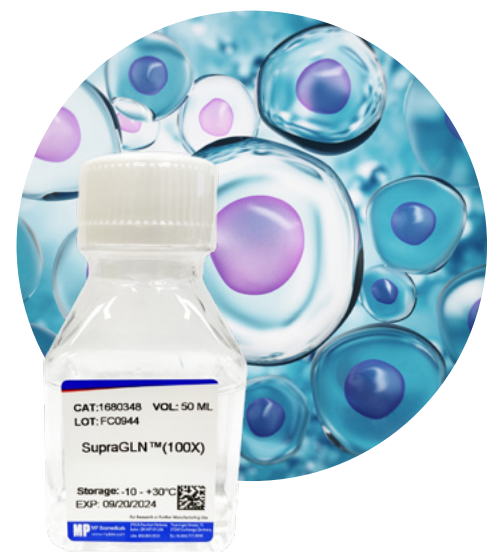
- Chemically defined nature without lot-to-lot variations
- No animal or human derived materials or compounds
- No interference with hormones or growth factors
- Elimination of the risk of contaminants – viruses, mycoplasma, prions, etc.
- Wide range of cell culture practices
- Storage in the refrigerator – no need for thawing before use

SupraGLN™ 100X, L-alanyl-L-glutamine 200 mM

SupraGLN™, a ALA-GLN (alanine-glutamine) dipeptide, has several advantages over traditional glutamine when used as a supplement in cell culture. One of the significant benefits of SupraGLN is its higher stability in solution compared to free glutamine. This improved stability enhances its shelf-life and reduces the need for frequent supplementation, making it more convenient for cell culture logistics.

Furthermore, SupraGLN is a more bioavailable form of glutamine, which means that it is more efficiently taken up by cells, leading to improved cell growth and proliferation. This increased bioavailability is due to its ability to be more rapidly and easily transported across cell membranes compared to free glutamine. The improved cellular uptake of SupraGLN can be particularly beneficial in conditions where cells have a high demand for glutamine, such as during periods of rapid cell growth or in response to stress. For example, immune-derived cells such as T cells, B cells, and macrophages, as well as cancer-derived cells and stem cells, can benefit from SupraGLN during cell growth, proliferation, differentiation, and overall cellular health.

Take your immune cell culture research to the next level with our high-grade expansion medium. We also offer custom manufacturing upon request for specific research requirements.



Learn more about our products for cell culture maintenance, expansion & treatment:

Name	Cat. No.
DMEM	
Dulbecco's Modified Eagle's Medium (DMEM), powder, with 4500 mg/L dextrose, L-glutamine, w/o sodium bicarbonate	0910331
Dulbecco's Modified Eagle's Medium (DMEM) (1X) with 20 mM HEPES, w/o L-glutamine or sodium bicarbonate	0912334
Dulbecco's Modification of Eagle's Medium (DMEM, 1X Solution) with L-glutamine and Sodium Pyruvate, and 1 g/L Dextrose	0912343
Dulbecco's Modified Eagle's Medium (DMEM) (1X Solution) w/o L-glutamine, phenol red, 500 mL	091642754
Dulbecco's Modified Eagle's Medium (DMEM), powder, with 4500 mg/L dextrose, L-glutamine, w/o sodium bicarbonate, sodium pyruvate, 10 x 1 L	091033220
Dulbecco's Modified Eagle's Medium with L-glutamine and 4.5 g/L glucose, 500 mL	091233354
RPMI	
RPMI 1640, with L-glutamine, w/o sodium bicarbonate	0910601
RPMI 1640 (1X) with L-glutamine with sodium bicarbonate	0912603
RPMI (1X) w/o L-glutamine, L-cysteine, L-cystine, and L-methionine, 500 mL	091646454
RPMI 1640 (1X) w/o L-glutamine, phenol red, 500 mL	091646754
RPMI 1640 with 2 g/L sodium bicarbonate, w/o L-glutamine & glucose, 500 mL	091646854
RPMI 1640 w/o L-glutamine, 6 x 500 mL	091260254X6
Animal & Serum Free FBS Replacement	
FastGro™ Synthetic, Chemically Defined FBS Replacement, Animal Free	0926400
Amino Acids	
SupraGLN™ 100X, L-alanyl-L-glutamine	0916803
L-glutamine	091680149
Non-essential amino acids for Minimum Essential Medium Eagle, 100X	091681049

CHAPTER 4

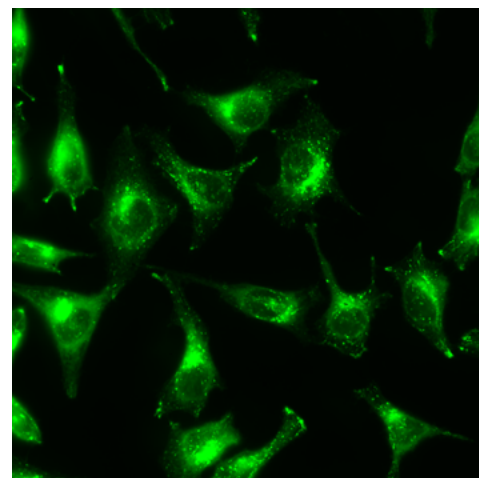
Cell-Based Assays

AUTOPHAGY, DNA DAMAGE, CELL IMAGING, AND NUCLEAR STAINING

Autophagy is vital in maintaining cellular homeostasis and preventing the development of diseases like cancer and neurodegeneration. Autophagy also plays a crucial role in regulating the innate immune response, including the phagocytic activity of macrophages and cytokine production by dendritic cells. Dysregulation of autophagy in white blood cells is implicated in immune-related disorders, such as inflammatory bowel disease and rheumatoid arthritis. Additionally, autophagy induction enhances antitumor activity, making it a potential therapeutic target in cancer immunotherapy. MP Bio's autophagy dye allows live or fixed cell imaging to track autophagy with the LC3 marker without transfection. It can assess cell survival in the tumor microenvironment, migration, invasion, chemotherapy resistance, and tumor escape from immunosurveillance.

Autophagy and Mitophagy Detection

MP Bio offers a cutting-edge autophagy and mitophagy detection kit that enables the characterization of autophagy and mitophagy levels in immune and tumor cells, providing valuable insights into tumor microenvironment cell survival, migration, invasion, chemotherapy resistance, and tumor escape from immunosurveillance.



Serum-starved HeLa cells with LC3 Analogue Dye 488G. HeLa cells were incubated with the dye for 30 minutes at 37 °C. Images obtained by fluorescent microscopy and autophagosomes are shown.

DNA Damage Detection

DNA damage is caused by both endogenous and exogenous factors, including metabolic processes, cellular activities, radiation, chemicals, and pollutants. This damage can lead to mutations, chromosomal aberrations, and cell death, which can have serious consequences for human health.

In cancer research, measuring DNA damage during drug discovery and pre-clinical stages can help determine a safety threshold for effective cancer treatment while minimizing toxicity to healthy cells.

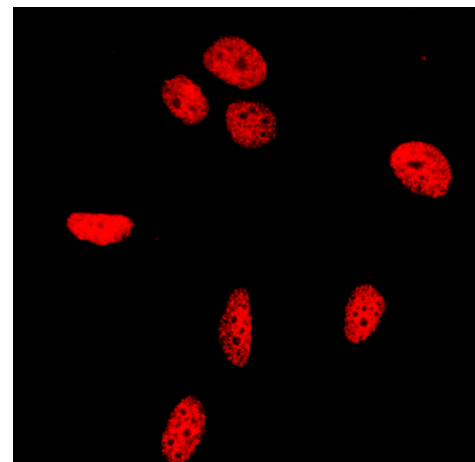
During bioprocessing, environmental stressors such as shear stress, high osmolality, and nutrient depletion can cause DNA damage, impacting cell viability and productivity. This can result in genetic changes in cells, affecting the stability of the final product.

MP Bio offers a DNA damage γ H2AX detection kit that provides direct visualization of the negative and positive effects of drugs during development and bioprocessing, helping to identify potential detrimental effects on a project.

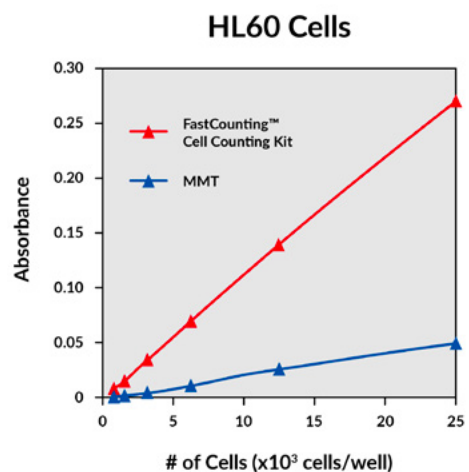
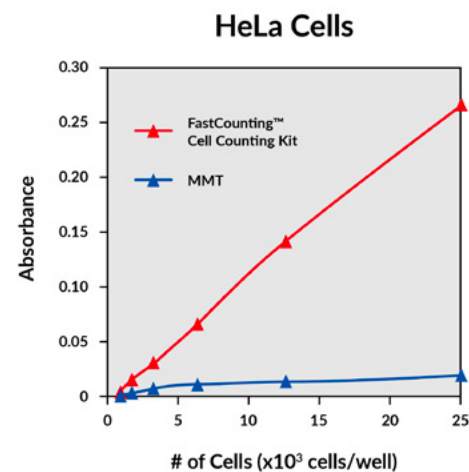
Detect high vs low cellular toxicity risks with MP Bio's DNA damage detection kits, assessing pre- and post-treatment DNA repair periods for accurate toxicity measurement.

Cell Viability

MP Bio provides various options for measuring cell viability based on the downstream application, sensitivity, specificity, and cell type. Our **FastCounting™** assay is a highly sensitive and convenient method for determining cell viability in cell proliferation and cytotoxicity assays. With the highest sensitivity dye for cell viability and the lowest cytotoxicity among tetrazolium reagents, our simple procedures can be performed without thawing reagents. In addition to our FastCounting assay, we also offer traditional reagents, such as trypan blue and MTT, for cases where high sensitivity is not required. Use our tools with confidence after any process of isolation, expansion, or treatment of blood-derived cells.

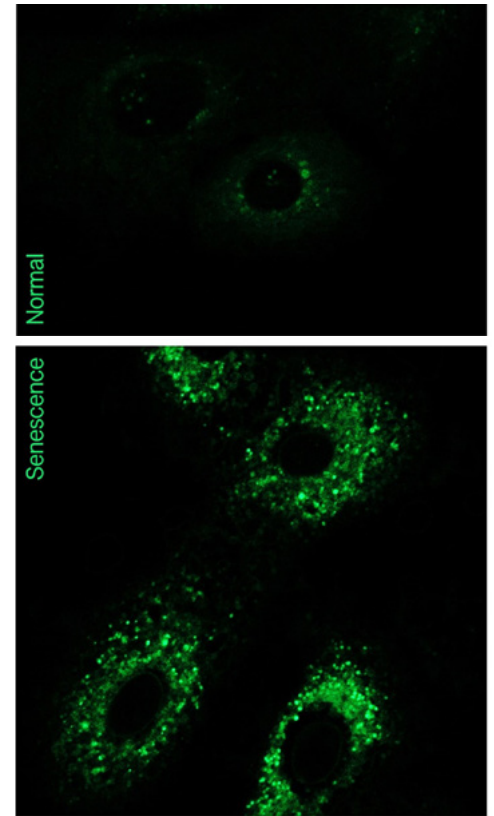


DNA damaged induced by UV irradiation for 30 minutes on HeLa cells.



Senescence Detection

We offer a sensitive and selective dye to measure senescence in blood-derived cells. Senescence can alter the gene expression and immune function of these cells due to factors such as aging, inflammation, cytokine storms, and oxidative stress. This can affect their response to treatment and the effective indication of drug discovery. Therefore, the senescence state of blood-derived cells plays a key role in age-related diseases and disorders. We also offer the traditional X-gal method for less time-sensitive applications.



Learn more about our cell-based assay products:

Name	Cat. No.
Mitophagy & Autophagy Detection	
FastMitophagy™ Detection Kit	092690201
LC3 Analogue Dye 488G Kit	092696600
LC3 Analogue Dye 700R Kit	092696700
DNA Damage Detection	
γH2AX Detection 488G Kit	092696300
γH2AX Detection 560R Kit	092696400
γH2AX Detection 650FR Kit	092696500
Cell Viability	
FastCounting™ Cell Counting Kit	09269013
MTT; 3-(4,5-Dimethylthiazolyl-2)-2,5-diphenyl tetrazolium bromide	02102227
Trypan blue, 0.4% solution in PBS, 100 mL	091691049
Senescence Detection	
FastCellular™ Senescence Detection Kit - SPiDER-βGal	092690301
X-Gal, powder	114063102



CHAPTER 5

DNA & RNA Extraction and Analysis

MP Bio specializes in providing tools and kits for DNA and RNA isolation from blood and blood-derived cells. Our products support research on genetic material from blood samples for studying disease mechanisms, drug response, and genetic variations.

Blood is a valuable source of genetic material for studying genetic mutations and variations that may be associated with disease risk or progression. For example, blood samples can identify specific mutations associated with cancer or other genetic diseases, enabling personalized treatment plans.

Gene expression and regulation can also be studied using blood DNA and RNA. Isolated white blood cells offer insights into molecular mechanisms behind biological processes, like immune response and inflammation, and help develop new therapies for related diseases.

Moreover, analyzing blood DNA and RNA can monitor drug response and toxicity by identifying the expression patterns of genes involved in drug metabolism and response. This information leads to more effective and personalized treatment strategies for various diseases.

Nucleic Acid Extraction

The **Rapid-DNA Blood/Cell/Tissue Kit** provides a fast and easy method for extracting DNA from whole blood, suspension or adherent cells, and various tissues. High quality genomic DNA (gDNA) is extracted and purified utilizing silica-based spin column technology, without the use of phenol or chloroform. The all-in-one kit includes optimized, individual protocols for blood, cells, and tissue samples for reproducible extraction of gDNA for downstream analysis.

We also offer a **RapidPure™ RNA Tissue Kit** for the isolation and purification of high-quality total RNA from small amounts of various human and animal tissues, tissues sections, and paraffin-embedded tissue samples.

Nucleic Acid Analysis

MP Bio has been an established manufacturer of PCR polymerases for over 20 years. We offer a range of high-quality PCR enzymes and master mixes that provide reproducible and consistent results for routine hot-start, high-fidelity, multiplex, and real-time PCR.

One of the areas where MP Bio has an extensive catalogue, as described here, is in blood-derived work. PCR-based detection of viral and bacterial infections in blood samples is a crucial tool for disease diagnosis. Our PCR enzymes and master mixes have been optimized to work with blood-derived DNA samples, enabling high efficiency and specificity in PCR amplification.

Moreover, MP Bio offers the **RapidScript™ cDNA Synthesis Kit**, which is engineered to provide high-performance cDNA synthesis from challenging RNA samples. The kit utilizes a specifically engineered enzyme ideal for blood-derived RNA samples, which often contain inhibitors that can interfere with the reverse transcription process.

Learn more about our products for DNA & RNA extraction and analysis:

Name	Cat. No.
Nucleic Acid Extraction	
Rapid-DNA Blood/Cell/Tissue Kit	116520005
FastDNA™ Kit	116540400
FastDNA™ SPIN Kit	116540600
RapidPure™ RNA Tissue Kit	112721050
Small RNAs	<i>Coming Soon!</i>
Nucleic Acid Analysis	
RapidTaq qPCR MasterMix (2X)	116187100
RapidTaq DNA Polymerase	116187200
RapidTaq PCR MasterMix (2X)	116187300
Rapid-RT MasterMix (5X)	116187400
RapidScript™ Hot-Start RT-Kit	116187500
RapidScript™ Hot-Start cDNA Synthesis Kit	116187800
RapidScript™ RT Kit	116187600
RapidScript™ cDNA Synthesis Kit	116187700

CHAPTER 6

Protein Analysis

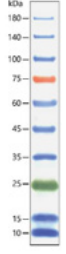
Protein analysis is used to demonstrate drug mechanisms of action and involves the detection, visualization, and quantification of a specific protein from a sample of tissue homogenate or extract. To achieve the desired results, several factors must be considered, including the technique itself and the quality of consumables utilized.

With the help of our reagents and consumables, PAGE analysis and Western blotting steps and procedures can be completed more quickly and effectively, producing better results in crucial areas such as:


- Accurate molecular weight measurements
- Improvements in gel staining
- Time-savings on blocking
- Enhanced signal detection
- Better band visualization

MP Bio also offers a range of reliable antibodies that can aid in identifying metastasis and immune response markers.

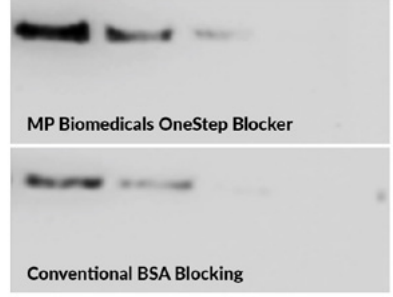
Band	Color	TRIS-GLYCINE/ kDa	BIS-TRIS (MOPS)/ kDa	BIS-TRIS (MES)/ kDa
1	Blue	180	170	170
2	Blue	140	130	130
3	Blue	100	93	93
4	Red	75	70	72
5	Blue	60	53	53
6	Blue	45	41	42
7	Blue	35	30	30
8	Green	25	22	23
9	Blue	15	14	14
10	Blue	10	9	10



Protein Extraction & Loading



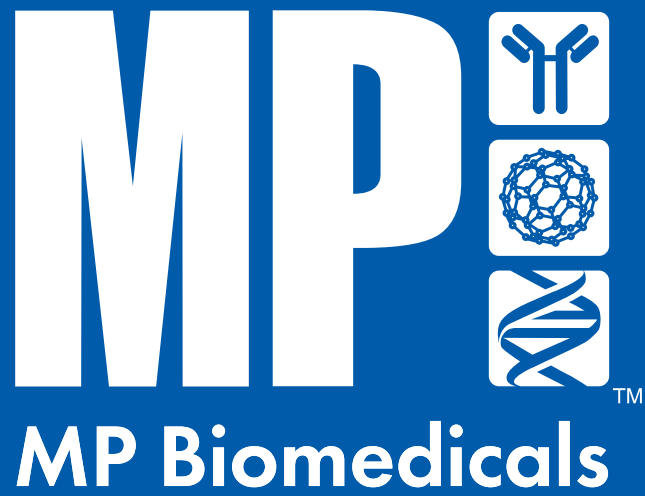
PAGE Analysis



Western Blot

Learn more about our products for protein analysis:

Name	Cat. No.
Western Blotting Reagents & Adjuvants	
Prestain Gel Plus, Tris Glycine	08W0000
Triple Color Precision Protein Ladder (10-180 kDa)	08L100025
Ultra-Blue Fast PAGE Gel Staining Solution	08X100001
OneStep Western Blocker and Signal Enhancer (Protein-Free)	08X100002
Ultra-Sense Femto-Plus Western ECL Substrate	08X100003
Antibody Diluent	08980641
FastAb Adjuvant	08642901
Freund's Incomplete Adjuvant	08642861
Vesicle Shedding & Metastasis	
CD63 Rabbit Monoclonal Antibody	08L100021
CD9 Rabbit Monoclonal Antibody	08L100023
CD81 Rabbit Monoclonal Antibody	08L100022
TSG101/VPS23 Rabbit Monoclonal Antibody	08L100024
Immune Response & Metabolism	
Human APO L1, Apolipoprotein L1, ELISA Kit	08L100040
Anti-human Complement C3 goat IgG fraction, FTIC	0855167
Anti-human Complement C3 goat IgG fraction, HRP	0855237
Anti-human Complement C4 goat IgG fraction, FTIC	0855168
Anti-human Complement C1Q goat IgG fraction, FTIC	0855166



MP BIOMEDICALS

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