

CANCER IMMUNOLOGY RESEARCH TOOLS

The immune system is central to many forms of cancer, being critical in both its development and its treatment. ATCC is supporting research into cancer immunology by offering a large collection of fully characterized and authenticated cell lines and human primary cells.

PRIMARY HUMAN IMMUNE CELLS

ATCC primary immune cells are able to support complex, physiologically relevant research projects, including:

- Cancer immunology studies
- Transplantation and graft rejection

Toxicity screening

- Inflammation and allergy
- Vaccine and drug development

ATCC scientists have conducted in-depth authentication and quality control analyses on each of the primary immune cell types. In addition, the utility of these cells for scientific studies has been confirmed by ATCC R&D scientists. For example, the differentiation capacity of the bone marrow CD34+ cells and the peripheral blood CD14+ monocytes was characterized. Additionally, to confirm their immune activity the peripheral blood CD56+ natural killer cells were utilized as the effector cells in an NK activity assay.



CONTINUOUS CELL LINES

ATCC houses a vast collection of cell lines derived from various normal and diseased tissues from multiple species, representing a variety of immunological cells. ATCC routinely authenticates its cell lines using the following methods:

- Short tandem repeat (STR) profiling, to establish a DNA fingerprint
- Cellular morphology, which is monitored for consistency
- Cytochrome C Oxidase I (COI) Assay, for species determination
- PCR testing, for mycoplasma detection

ATCC offers many cells and other products for cancer immunology research. To see ATCC's complete cancer immunology offering, please visit <u>www.atcc.org/cancerimmunology</u>

HUMAN PRIMARY CELLS FOR CANCER IMMUNOLOGY

ATCC maintains a number of primary immune cells which are relevant to cancer research and immunotherapy development. Primary cells from ATCC further help researchers obtain clear, confident results by increasing the physiological relevance of their assays.

Positive and negative biomarkers

Greater than 80% cryo-recovery

Normal cell morphology

- High differentiation capacity or immune activity
- Expansion or differentiation protocols
- Bio-functional data for select cell types
- Greater than 90% purity for select biomarkers
- Diverse pool of donors

PRIMARY MONONUCLEAR CELLS

Mononuclear cells, comprising monocytes and lymphocytes, are critical parts of both the natural immune response to cancer as well as immunotherapies. Mononuclear cells include terminally differentiated and undifferentiated immune cells commonly used in the study of immune regulatory processes in cancer immunology.

PRIMARY LYMPHOID CELLS

Lymphoid cells provide long-lasting immunity against microbes and pathogens, and play important roles in disorders such as autoimmune disease, immunodeficiency, and cancer. Lymphoid cells, the primary effector cells of the adaptive immune system, are critical to the development of cancer immunotherapies.

PRIMARY PROGENITOR CELLS

All forms of mature blood cells must be regenerated throughout the lifespan of an organism. Hematopoietic progenitor cells self-renew and are capable of differentiating into all blood cell types. Understanding progenitor cells and the cells that derive from them is critical to many fields, including hematology, immunology, oncology, and others.

www.atcc.org/mononuclear



www.atcc.org/lymphoid

www.atcc.org/progenitor





Table 1: Human primary cells for cancer immunology

ATCC [®] No.	Designation	Cells/vial	Positive Biomarkers
Mononuclear cells			
<u>PCS-800-010</u> ™	Peripheral Blood CD14 Monocytes	50 million	CD14, CD45
<u>PCS-800-011</u> ™	Peripheral Blood Mononuclear Cells	25 million	CD45
<u>PCS-800-013</u> ™	Bone Marrow Mononuclear Cells	25 million	CD45
Lymphoid cells			
<u>PCS-800-016</u> ™	CD4+ T Cells	25 million	CD3, CD4, CD45
<u>PCS-800-017</u> ™	CD8+ T Cells	25 million	CD3, CD8, CD45
<u>PCS-800-018</u> ™	CD19+ B Cells	25 million	CD 20, CD45
<u>PCS-800-019</u> ™	CD56+ Natural Killer (NK) Cells	5 million	CD45, CD56
Progenitor cells			
<u>PCS-800-012</u> ™	Bone Marrow CD34+ Cells	500,000	CD34, CD45
PCS-800-014 [™]	Cord Blood CD34+ Cells	500,000	CD34, CD45

NATURAL KILLER ACTIVITY ASSAY USING PRIMARY CD56+ NK CELLS AND A K562-GFP REPORTER CELL LINE

Natural Killer (NK) Cells were first identified for their ability to kill tumor cells without activation. NK Cells are a focus of many cancer immunotherapies, as they display rapid and potent immunity to metastasis or hematological cancers.

Measuring the activity of natural killer cells is a necessary goal in many immunological applications, however such assays are historically difficult to perform. The most commonly used method, the radioactive ⁵¹chromium (⁵¹Cr) release assay, is expensive, requires specialized equipment, has regulatory hurdles, and suffers from a short reagent half-life and generation of hazardous waste. Alternatives utilizing flow cytometry with fluorophore-labeled target cells using fluorescent dyes suffer from high variability due to inconsistent dye uptake and spontaneous dye leakage over the course of the assay.

Primary CD56+ NK Cells (ATCC[®] <u>PCS-800-019</u>[™]) are isolated from mononuclear cells by negative selection using immunomagnetic cell separation procedures. These cells are shown by flow cytometry for the expression of CD45+, CD56+, CD56+CD3-, and CD56+CD16+. K-562-GFP (ATCC[®] <u>CCL-243-GFP</u>[™]) cells utilize a clonal version of the immortalized myelogenous leukemia line K-562 engineered to express GFP. This K-562-GFP[™] line can be incorporated into cytotoxicity assays with no upstream preparation time and addresses all the drawbacks of previous methods.



Figure 1: Cytotoxic Killing Activity in ATCC Natural Killer Cells.

Three lots of human natural killer cells (ATCC[®] <u>PCS-800-019</u>[™]) were tested for cytotoxic killing activity using GFP expressing K-562 cells in a flow cytometry-based assay. Depending on the effector:target ratio and the donor, the extent of lysis ranged from 4% to a maximum of 54%.

MONOCYTE ACTIVATION TEST USING PERIPHERAL BLOOD MONONUCLEAR CELLS AS A SUBSTRATE

Primary human peripheral blood mononuclear cells (PBMCs) are needed as indicators in certain cell-based assays such as monocyte activation tests (MATs); PBMCs are a source of immune cells that, in response to pyrogens, secrete the pro-inflammatory cytokines that are detected by a MAT. In vivo, these pro-inflammatory cytokines can cause fever, shock, and death. Thus, the detection of these cytokines is critical to identifying the presence of pyrogenic compounds or organisms in medical devices and drug delivery systems to ensure the safety of the consumer. Primary Human PBMCs (ATCC[®] <u>PCS-800-011</u>[™]) can be frozen in liquid nitrogen and stored almost indefinitely. PBMCs respond to the presence of pyrogens such as lipopolysaccharide (LPS) and lipoteichoic (LAT) by secreting cytokines such as interleukin-6 (IL-6), which can then be detected by a simple, rapid ELISA-based test.





Figure 2: IL-6 activation in PBMC treated with pyrogens. A) PBMCs were incubated with the indicated concentrations of LPS for 24 hours at 37 °C in a 96-well plate. The supernatant from each well was then transferred into an IL-6-coated 96-well plate and an ELISA was performed. Increased IL-6 was detected at 0.125 EU LPS; the IL-6 activation was saturated with 1.0 EU LPS. B) PBMCs were incubated with the indicated concentrations of LTA for 24 hours at 37 °C in a 96-well plate and an IL-6 ELISA was performed as previously stated. Increased IL-6 was detected at 0.5 µg LPS; the IL-6 activation nearly saturated with 50 µg of LTA.

ATCC [®] No.	Designation	Species; Comments	
<u>CRL-2003</u> ™	TF-1	Homo sapiens; bone marrow erythroleukemia	
<u>CRL-10423</u> ™	JM-1	Homo sapiens; B cell lymphoma	
<u>CCL-243</u> ™	K-562	Homo sapiens; chronic myelogenous leukemia	
<u>CRL-1593.2</u> ™	U-937	Homo sapiens; pleural effusion, lymphocyte, myeloid	
<u>CCL-213</u> ™	Daudi	Homo sapiens; peripheral blood, B lymphoblast	
<u>TIB-152</u> ™	Jurkat, Clone E6.1	Homo sapiens; acute T cell leukemia	
<u>TIB-202</u> ™	THP-1	Homo sapiens; acute monocytic leukemia	
<u>TIB-71</u> ™	RAW 264.7	Mus musculus; murine leukemia virus-induced tumor	
<u>CCL-243-GFP</u> ™	K562-GFP	Homo sapiens; chronic myelogenous leukemia	

Table 2: Cell Lines for Cancer Immunology

ATCC offers many other cell lines applicable for cancer immunology research. For a complete list of relevant cell lines, please visit www.atcc.org/cancerimmunology

CELL HEALTH DETECTION KITS

Precise and sensitive methods for assaying the heath of cell cultures are essential to life science research. ATCC has developed kits and reagents to assess the growth and viability of your cells, including:

MTT Cell Proliferation Assay (ATCC[®] <u>30-1010K</u>[™])

Mycoplasma contamination constitutes a serious concern for cell culturists as it can result in a number of deleterious effects, which in turn can affect assay reproducibility, compromise data validity, and lead to the misinterpretation of results. We provide an easy-to-use PCR-based assay the Universal Mycoplasma Detection Kit (ATCC[®] 30-1012K[™]) to detect contamination by this opportunistic pathogen.

For more information, www.atcc.org/cellhealth

ADDITIONAL CANCER IMMUNOLOGY RESOURCES

- 1 Ap Note: A Simple and Rapid Alternative to ⁵¹Chromium or Fluorescent Dye Loading for Quantification of Natural Killer Cell Activity: ATCC[®] K-562-GFP[™] Cells
- 2 Ap Note: Differentiation and Expansion of Hematopoietic Precursor Cells from Bone Marrow-Derived CD34+ Progenitors
- 3 Ap note: In Vitro Differentiation of Macrophages and Dendritic Cells from Primary Human CD14+ Monocytes
- 4 Webinar: Discovering ATCC Hematopoietic Progenitor Cells Model Systems to Study the Immune and Cardiovascular Systems
- 5 Poster: Using Tumor Cell Panels in Drug Discovery: New Tools for the Genomic Era



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