

# Stem Cell Research Source

## From Isolation to Culture and Analysis



Helping all people  
live healthy lives



# Few scientific breakthroughs have sparked as much interest, imagination, and hope as the isolation and successful in vitro culture of human pluripotent stem cells less than a decade ago.

Recent discoveries in stem cell biology have both amplified the importance of pluripotent stem cells in therapeutics and improved researchers' understanding of both normal and disease processes. Based on what is known today, the continuing study of embryonic, adult, and induced pluripotent stem cells will ultimately allow researchers to understand how cells differentiate into all body cell types. Central to the tools and applications used to assess the function of the many differentiated cell lineages are the methods initiated for the successful isolation and characterization of homogeneous cell populations.

First to commercialize systems and reagents for characterizing and isolating hematopoietic stem cells for research use, BD Biosciences has more than 25 years of experience supporting stem cell research. Today, BD Biosciences offers a diverse set of tools including flow cytometry instrumentation, multicolor reagents, cell culture environments, and bioimaging technology to support the expanding efforts in research and discovery. This ever-evolving tool set combines the power of advanced technologies and world-class services to support investigative efforts across stem cell isolation, culture, and analysis.

# BD Biosciences

A Singular Resource  
for Stem Cell Research

## Advanced Tools for the Isolation, Culture, and Analysis of Stem Cells

BD has provided integrated, high-value products and services for stem cell culture and analysis for more than two decades. Today, this tradition continues with one of the most comprehensive solution sets available for stem cell researchers. Across stem cell isolation, culture, and analysis, BD Biosciences product offerings continue to grow with new instruments, cultureware, and reagents on the leading edge of biomedical discovery.

## BD Flow Cytometry Systems

In stem cell research, biomarkers are critical to the study of cellular differentiation and cell-fate specification. To study the transition from one cell type to another, researchers must conclusively identify distinct cell types. Cell identification can be accomplished quickly and accurately using flow cytometry. A cell sorter can rapidly separate the rare stem cells from the millions of other cells present in a tissue. Using flow cytometry, researchers can sort on a number of different parameters simultaneously—including more than one type of marker. This is important since a number of molecular markers can be used to characterize stem cell populations, and combinations of markers are sometimes used to identify a particular type of stem cell.

## BD High-Content Cell Imaging Systems

BD offers high-content cell imaging instruments together with bioimaging-certified reagents to form a validated platform for your stem cell research applications. The rapidly growing collection of antibody reagents available from BD Biosciences has been tested and certified for utility in automated bioimaging (high-content screening), and fluorescence microscopy applications. Bioimaging-certified reagents from BD Biosciences are optimal for use in high-content applications for which reproducibility is essential. Together with our state-of-the-art bioimaging instrumentation, we now offer a unique platform to support your stem cell research.

### Cell Culture Tools and Environments

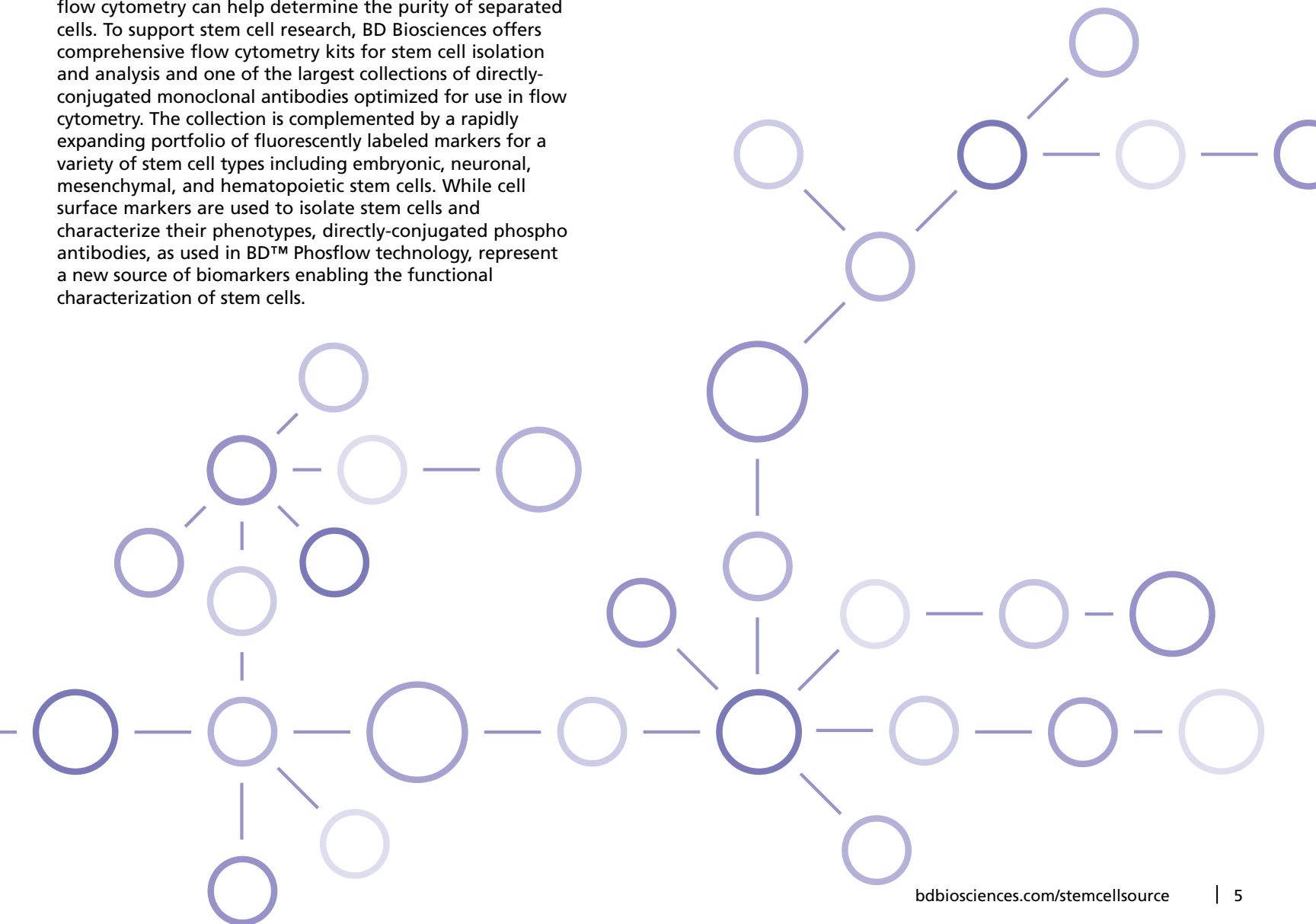
Once stem cells are isolated, subsequent expansion and manipulation in cell culture remains challenging. Successful culture and differentiation require specific surfaces, cytokines, and media additives. BD Falcon™, BD BioCoat™, and BD Extracellular Matrix proteins provide the appropriate surfaces for the attachment and differentiation of various cell types. Working with industry leaders, BD Biosciences has pioneered the development of optimized feeder-independent environments for human embryonic stem cells.

### High-Quality Reagents

In addition to its use for highly specific stem cell isolation, flow cytometry can help determine the purity of separated cells. To support stem cell research, BD Biosciences offers comprehensive flow cytometry kits for stem cell isolation and analysis and one of the largest collections of directly-conjugated monoclonal antibodies optimized for use in flow cytometry. The collection is complemented by a rapidly expanding portfolio of fluorescently labeled markers for a variety of stem cell types including embryonic, neuronal, mesenchymal, and hematopoietic stem cells. While cell surface markers are used to isolate stem cells and characterize their phenotypes, directly-conjugated phospho antibodies, as used in BD™ Phosflow technology, represent a new source of biomarkers enabling the functional characterization of stem cells.

### Services

BD Biosciences is fully committed to the success and satisfaction of its customers. A world-class service and support organization offers customers knowledge and expertise to help optimize and advance their research through comprehensive training, applications and technical support, and expert field service and custom services.



# Hematopoietic Stem Cells

Historically, many of the earliest methods and attempts to isolate progenitor subsets from blood and bone marrow involved separating cells based upon size and cell cycle characteristics. However, these techniques were not sufficient for successful analysis.

Early transplantation studies of bone marrow derived hematopoietic stem cells (HSCs) revealed the need to qualitatively and quantitatively measure the impact and success of transplantation. Multiple cellular markers, including intracellular proteins and cell surface receptors, were analyzed to distinguish host cells from graft cells.

The development of the fluorescence-activated cell sorter and the availability of monoclonal antibodies to cell surface markers have made it possible to analyze and isolate hematopoietic stem cells from heterogeneous cell populations.

Despite these breakthroughs, the field of hematopoietic stem cell research was slowed by the lack of a single biomarker to distinguish different stem cell subpopulations, and specifically mark the pluripotent cell population.

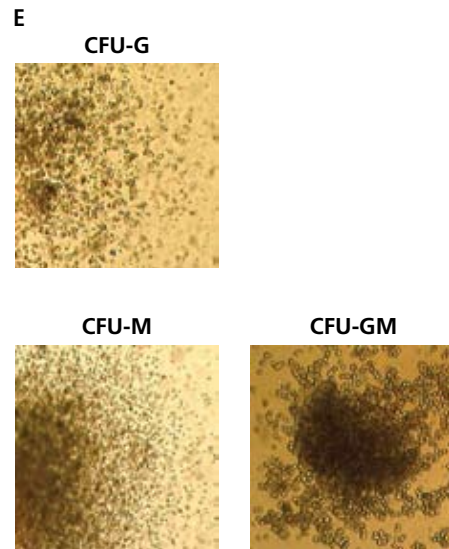
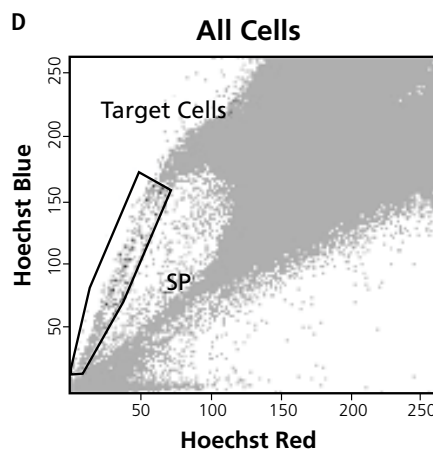
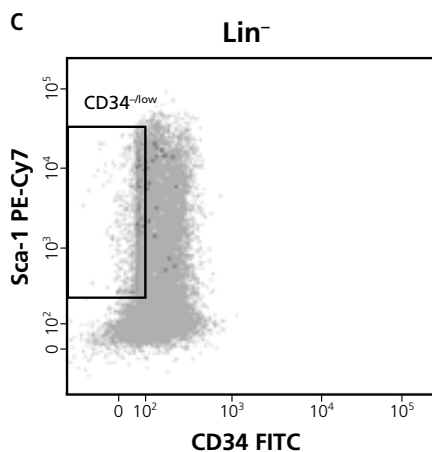
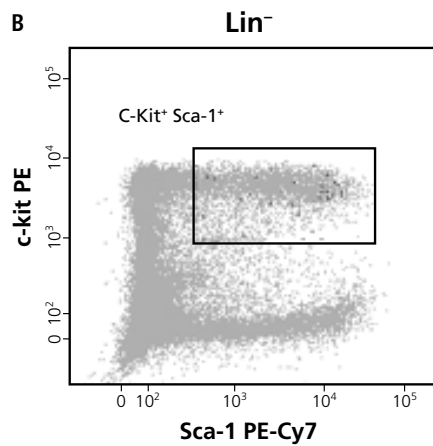
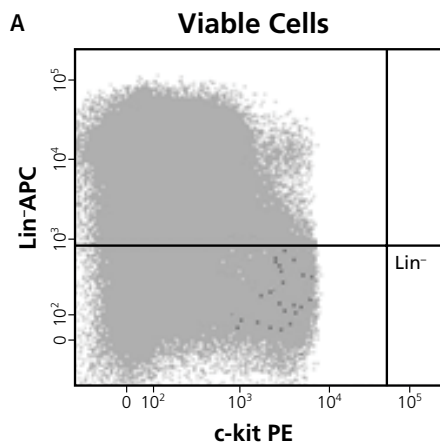
Today, numerous cell-surface-specific antibodies, dyes, and fluorophores are available for use with multiparameter flow cytometric approaches for defining different stem cell lineages.

HSCs are found in the bone marrow of adult femurs, hips, and other bones. Cells can be obtained directly by removal from the bone or from the blood following pretreatment with cytokines, such as G-CSF (granulocyte colony-stimulating factors). Other sources for clinical and scientific use include umbilical cord blood and placenta. For experimental purposes, fetal liver and fetal spleen of animals also are useful sources of HSCs.

## Tools for Stem Cell Isolation

BD Biosciences offers two advanced flow cytometry systems for single-platform, high-purity cell sorting and multiparametric analysis, the BD FACSAria™ II and the BD Influx™ cell sorting systems. To further reduce sorting times of rare subpopulations of cells such as mouse hematopoietic progenitor cells from bone marrow, BD IMag™ cell separation products can be used to deplete lineage-committed cells from samples in preparation for isolation by flow cytometry.





**Identification and enrichment of adult mouse hematopoietic stem cells with the BD FACSAria II cell sorter. Side population (SP) analysis of CD34<sup>-</sup>, c-kit<sup>+</sup> (CD117), Sca-1<sup>+</sup>, Lin<sup>-</sup> (CD34KSL) cells in mouse bone marrow (BM). Flow cytometric analysis of CD34 KSL cells stained with Hoechst 33342 is shown.**

**A** Unfractionated mouse BM cells with the sorting gate set for Lin<sup>-</sup> cells.

**B** Lin<sup>-</sup> cells are displayed for Sca-1 and c-kit expression with the sorting gate set for Sca-1<sup>+</sup>, c-kit<sup>+</sup> cells.

**C** CD34 expression of KSL with the sorting gate set for the CD34<sup>-</sup> cell population.

**D** The CD34<sup>-</sup> cells displayed with Hoechst Red and Blue dyes. This CD34 KSL/SP subset of cells makes up approximately 0.0025% of the nucleated cells found in BM.

**E** To assess functionality of sorted cells, mouse colony-forming cell (CFC) assays were performed using Methocult™ GF M3534 medium, which has been formulated to support optimal growth of granulocyte (G) and macrophage (M) progenitor colonies. Representative colony-forming units (CFUs) are displayed. The following antibodies were used for sorting: CD34 FITC (Cat. No. 560238), CD117 c-kit PE (Cat. No. 553355), LY-6 A/E Sca-1-PE-Cy7™7 (Cat. No. 558162), and Lin<sup>-</sup> cocktail APC (Cat. No. 558074). Cells were sorted on a BD FACSAria II flow cytometer.

### BD FACSAria II cell sorting system

The BD FACSAria II system makes high-speed, multicolor cell sorting easy and accessible to life science researchers working across a wide range of advanced applications. The BD FACSAria design is based on a true fixed-alignment flow cell that is gel-coupled to the collection optics. This unique and revolutionary design minimizes startup time, improves experiment-to-experiment reproducibility, and increases the sensitivity and resolution needed for multicolor applications. A simple fluidics design allows for easy aseptic cleaning. A near-UV laser option further supports the isolation and analysis of side populations, an application used to enrich stem cells from mixed-cell populations.

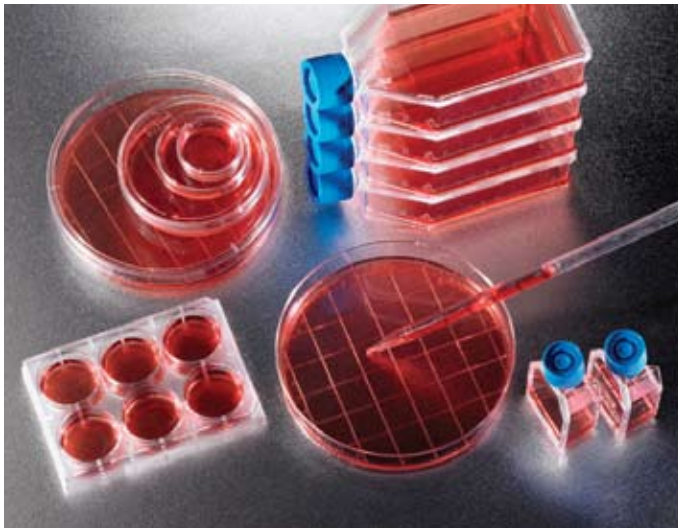
### BD Influx cell sorting system

The BD Influx cell sorter is an open, configurable cell sorting platform that offers a powerful combination of detection capability, sorting performance, and hands-on control. This compact instrument features 5 laser ports, replaceable nozzle assemblies, gamma-irradiated quick-exchange fluidics and tubing, and an optional HEPA-filter enclosure. The BD Influx cell sorter can be fully optimized for routine tasks or emerging applications including cell therapy research, stem cell research, and drug discovery.



# Embryonic Stem Cells

Over the last decade, interest has rapidly grown beyond hematopoietic stem cells to understand the role of embryonic and other adult (or somatic) stem cell populations.



BD Falcon cultureware

Research in embryonic and adult stem cell populations has unique challenges in manipulating the growth conditions and downstream identification, isolation, and purification of the myriad of progenitor stem cell networks. Each of these distinct stem cell types exhibits a unique set of challenges in understanding cell growth and differentiation.

Embryonic stem cells (ESCs) are pluripotent and can differentiate into all derivatives of the three primary-germ layers: ectoderm, endoderm, and mesoderm. The presence of pluripotent adult stem cells remains a subject of scientific debate. However, research has demonstrated that pluripotent stem cells can be directly generated from adult fibroblast cultures. These induced pluripotent stem (iPS) cells, such as ESCs, can form all three germ layers and self-renew.

Tremendous hope is associated with the potential application of ESCs and iPS cells in cell therapy and regenerative medicine because of their ability to differentiate into multiple, clinically-useful cell types. Defined culture conditions, high-affinity antibodies, and the appropriate analysis tools are essential for realizing the potential of ESCs and iPS cells.

## Tools for Stem Cell Culture

For more than 25 years, BD's extensive experience in the development of cell culture environments, along with rigorous quality assurance testing, has guaranteed high-quality, consistent products.

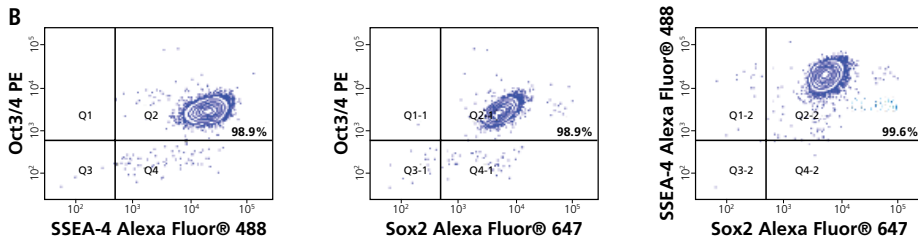
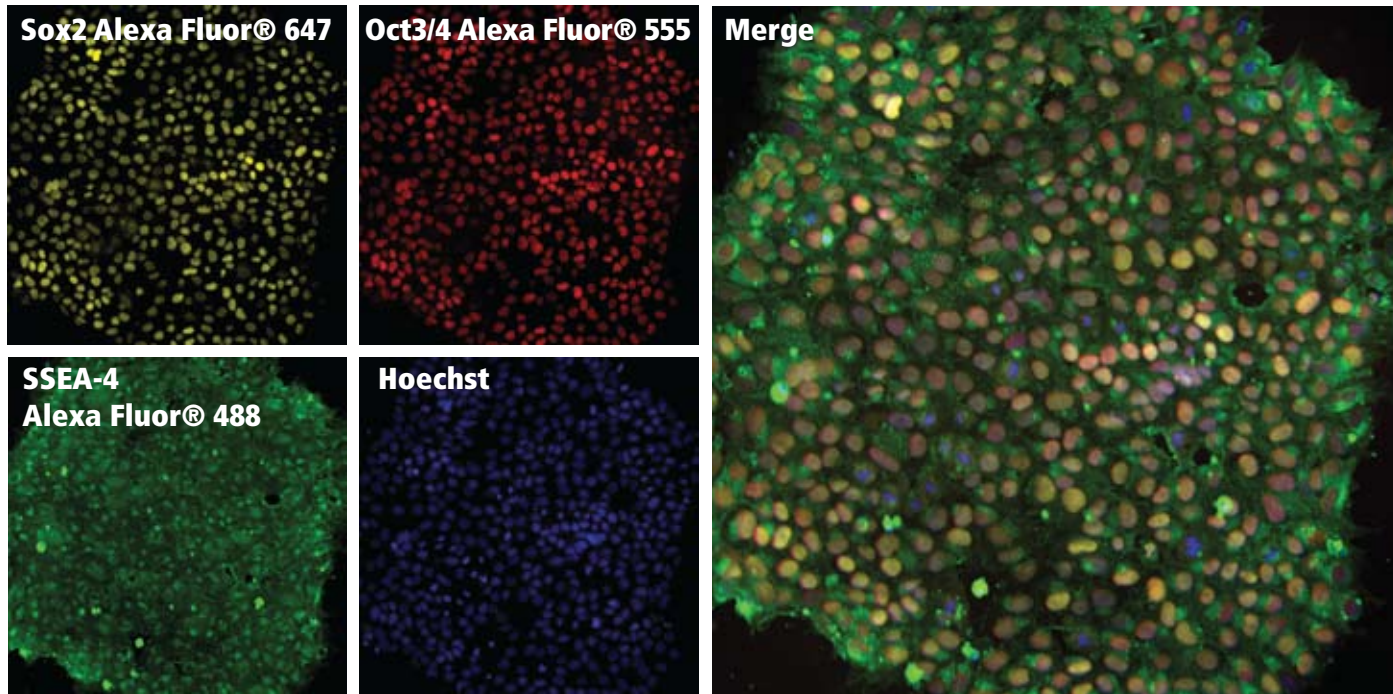
## Feeder-independent culture tools and growth factors

For culture of hESCs, BD Matrigel™ matrix and BD™ Laminin/Entactin High Concentration-coated surfaces eliminate maintenance of a second cell type and cross-contamination of hESC samples with feeder-cell-derived RNA and DNA. To give researchers more time for experiments, BD, in collaboration with StemCell Technologies and WiCell™ Research Institute, provides a complete, pre-qualified environment for culturing hESCs comprised of BD Matrigel hESC-qualified matrix and mTeSR™1, a completely defined medium.

BD extracellular matrix proteins (ECMs), cytokines, and growth factors provide a broad range of reagents for more defined cell culture and differentiation conditions to facilitate consistent, reproducible results.



A



**Embryonic stem cells cultured under feeder-free conditions remain pluripotent.**

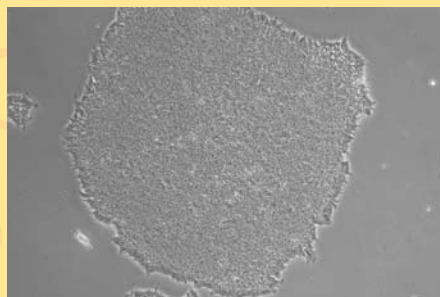
**A** Human embryonic stem cells (H9) were cultured in mTeSR1 maintenance medium (StemCell Technologies) on BD Falcon 96-well imaging plates (Cat. No. 353219) that were coated with BD Matrigel hESC-qualified matrix (Cat. No. 354277). Cells were fixed with 4% paraformaldehyde, followed by BD Perm/Wash™ buffer (Cat. No. 554723). Multicolor cell staining was performed with the following antibodies: Sox2 Alexa Fluor® 647 (Cat. No. 560302) pseudocolored yellow,

Oct3/4 Alexa Fluor® 555 (Cat. No. 560306) pseudocolored red, and SSEA-4 Alexa Fluor® 488 (Cat. No. 560308) pseudocolored green. Cell nuclei were counterstained using Hoechst 33342 pseudocolored blue. Small images on the left show individual antibody staining. The larger panel on the right represents the merged image. The cells were imaged on a BD Pathway™ 435 bioimager using a 10x objective.

**B** Human embryonic stem cells (H9) were grown in 6-well culture plates coated with BD Matrigel hESC-qualified matrix and mTeSR1 medium. Cells were dissociated into a single cell suspension and then fixed with 4% paraformaldehyde, followed by BD Perm/Wash buffer. Cells were incubated with the same cocktail of fluorochrome-conjugated antibodies used in the imaging experiments described in A except that Oct3/4 PE (Cat. No. 560186) was used in place of Oct3/4 Alexa Fluor® 555. Cells were run on a BD™ LSR II and data was analyzed using BD FACSDiva™ software.

**High-quality, synthetic and biological cultureware**

BD offers a range of cultureware, from synthetic to biological coatings, to meet the needs of many cell types. BD Falcon™ cultureware offers consistent tissue culture surfaces for use with a broad range of applications. BD BioCoat™ cultureware is pre-coated with biological and synthetic attachment factors, designed to mimic in vivo environments and to maximize cellular activity, delivering reproducible results in a broad range of research applications. Membrane inserts provide a more natural cell growth environment by allowing cells to access media from both their apical and basolateral sides, facilitating the study of paracrine interactions, cell migration, and invasion.



**Embryonic stem cells cultured under feeder-free conditions**  
A bright field image of H9 hESCs cultured in mTeSR1 maintenance medium (StemCell Technologies) on BD Matrigel hESC-qualified matrix (Cat. No. 354277) coated BD Falcon tissue culture plates.

## Neural Stem Cells

Differentiation of embryonic stem cells (ESCs) towards different lineages is routinely measured by morphology and expression of both intracellular and cell surface markers. Understanding the cell surface marker signatures of stem cells and their differentiated progeny is crucial for the enrichment of a particular cell type from a heterogeneous pool.

Neural stem cells (NSCs), the multipotent self-renewing cells that generate neurons and glia, are a main area of focus in stem cell research.

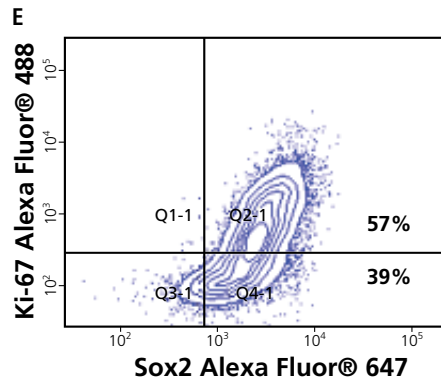
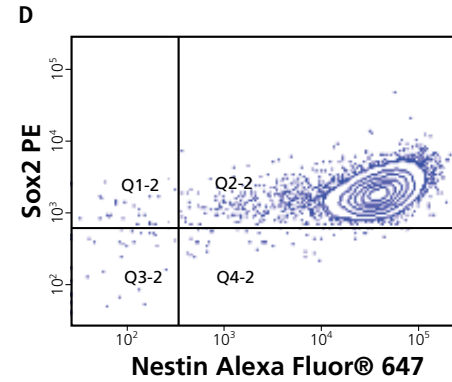
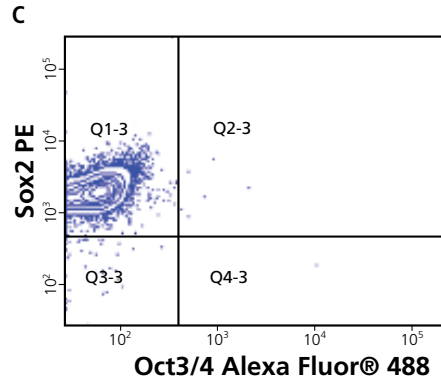
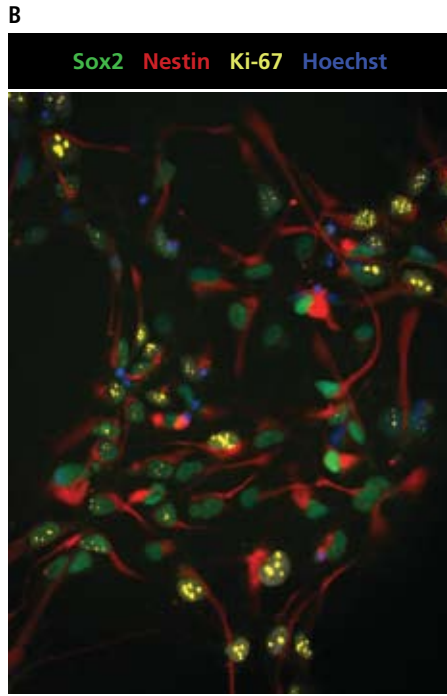
Unlike in other tissues, active cellular turnover does not occur in the adult nervous system. For this reason, it was historically postulated that the adult brain and spinal cord were unable to regenerate new nerve cells. However, since the early 1990s, NSCs have been successfully isolated from brain tissues. Found in the subventricular zone lining the lateral ventricles and in the hippocampus of the adult brain, NSCs are able to divide and give rise to nerve cells (neurons) and neuron-supporting cell types. Their remarkable discovery is the focus of both popular and scientific interest because of their potential impact on human health.

### Tools for Stem Cell Analysis

BD flow cytometry analyzers can be used in combination with fluorochrome-conjugated antibodies to count members of cell subpopulations and obtain data on the relative expression level of multiple markers for individual self-renewing or differentiated cells.

BD high-content cell imagers, together with BD Bioimaging certified reagents, enable researchers to observe and quantify multiple cellular processes that include cell cycle, proliferation, apoptosis, and differentiation. Applications range from drug discovery, where high throughput is key, to basic research, where flexibility is a necessity.





**F**  
Sox2: hESCs, NSCs  
Nestin: NSCs  
Oct3/4: hESCs  
Ki-67: Proliferation  
Hoechst: Cell nuclei

**Analysis of hESC-derived self-renewing neural stem cells (NSCs) by bioimaging and flow cytometric analysis.**

**A** Timeline for differentiating human embryonic stem cells (hESCs) into self-renewing neural stem cells.

**B** Human embryonic stem cell-derived neural stem cells were cultured on BD Falcon 96-well imaging plates (Cat. No. 353219) that were coated with poly-L-ornithine and laminin. Cells were fixed with 4% paraformaldehyde, followed by BD Perm/Wash buffer (Cat. No. 554723). Multicolor cell staining was performed with the following antibodies: Ki-67 Alexa Fluor® 488 (Cat. No. 558616)

pseudocolored yellow, Sox2 Alexa Fluor® 647 (Cat. No. 560294) pseudocolored green, and Nestin Alexa Fluor® 555 pseudocolored red.

Cell nuclei were counterstained using Hoechst 33342 pseudocolored blue. The cells were imaged on a BD Pathway 435 bioimager using a 20x objective.

**C, D** Neural stem cells were dissociated and fixed using BD Cytofix/Cytoperm™ fixation/permeabilization solution (Cat. No. 554722), then washed using BD Perm/Wash buffer. Cells were

stained with Sox2 PE (Cat. No. 560291), Nestin Alexa Fluor® 647, and Oct3/4 Alexa Fluor® 488 (Cat. No. 560253) antibodies. Purity of neural stem cells is approximately 99% co-expressing Nestin and Sox2.

**E** Samples were prepared as described in C, D. Cells were stained with Ki-67 Alexa Fluor® 488 and Sox2 Alexa Fluor® 647 and then run on a BD FACSCanto™ II cytometer and analyzed with BD FACSDiva software.

**F** Key to markers and cell types recognized.

**BD LSR cell analyzers**

The proven BD™ LSR cell analyzers can be configured to meet exact lab and assay requirements and easily upgraded to meet future needs. The special order BD LSR II has the capacity to support up to 7 lasers and has 56 positional choices for selection of the detectors. The special order BD LSRFortessa™ cell analyzer delivers this power in a benchtop fit that is 50% smaller, yet it offers a wide range of choices—including 5 lasers, 30 detector positions, and 11 laser wavelengths. Both of these LSR cell analyzers can detect up to 18 colors simultaneously.

**BD Pathway 855 high-content analyzer**

The BD Pathway™ 855 system offers the flexibility for high-content imaging of live and fixed cells. Equipped with environmental control and liquid handling, the system has full-spectrum (340–700 nm) illumination, laser auto focus, and fast filter changers. These powerful features, along with spinning disk confocal optics and a cooled CCD camera, enable the BD Pathway 855 system to rapidly record high resolution fluorescence images from multiwell plates and slides.

**BD Pathway 435 high-content analyzer**

A compact benchtop analyzer for high-content cellular imaging, this system is ideally suited for endpoint biological assays. Light from a mercury metal halide lamp, introduced through a liquid light guide, provides full-spectrum illumination. The laser auto focus, fast filter changers, spinning disk confocal optics, and a high-resolution CCD camera enable the system to record high-quality fluorescence images from multiwell plates and slides. A transmitted light canopy captures bright field images to overlay on fluorescence images.

# Mesenchymal Stem Cells

The study of stem cells offers a new way to better understand the fundamental events and mechanisms regulating human health, development, and disease.

The ultimate goal of stem cell research is to develop cell-based therapies to treat conditions currently untreatable by conventional therapies. To achieve this, protocols are needed to expand, characterize, and isolate sufficient quantities of purified cell populations of a defined lineage.

Today the importance of the purity of the cell population to patient survival has been proven in stem cell transplantation procedures. Flow cytometry, combined with the development of new antibodies, can improve cell population purity and provide faster multiparametric analysis to help accelerate the pace of discovery.

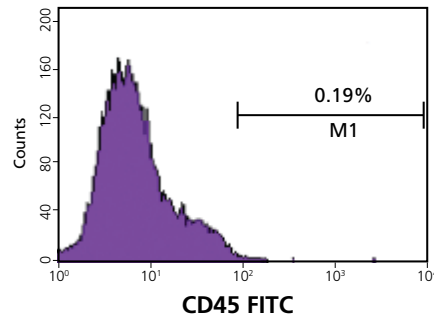
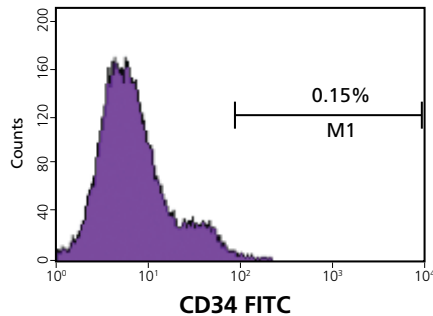
Mesenchymal stem cells (MSCs) are thought to have promising potential in therapeutics. MSCs have been reported to differentiate into a variety of cell types such as osteoblasts, chondrocytes, myocytes, adipocytes, and pancreatic beta-islet cells and neuronal cells. MSCs are multipotent stem cells found in bone marrow. They have also been isolated from adipose tissue, umbilical cord, synovium, amniotic fluid, and dental pulp.

BD Biosciences has one of the largest collections of reagents for flow cytometric analysis, bioimaging, immunofluorescence microscopy, cytometric bead arrays (CBA), ELISA, and Western blot analysis. These reagents include a rapidly expanding portfolio of BD stem cell kits for the high-purity isolation and in-depth analysis of stem cells by flow cytometry. For cell signaling analysis of self-renewal and differentiation, BD Phosflow products including conjugated antibodies, buffers, and supporting protocols enable biochemical access to rare cell subsets and functional characterization at the single-cell level.

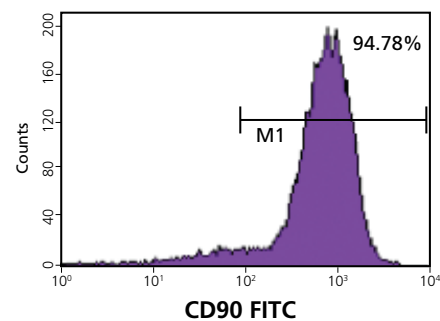
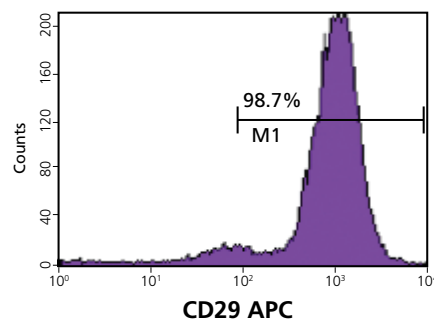
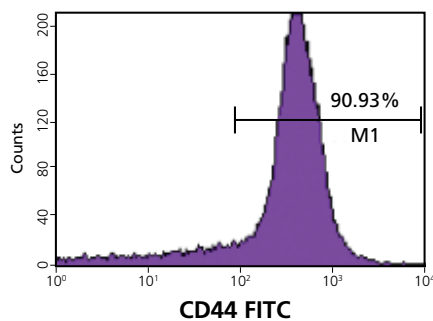




## Negative Markers



## Positive Markers



### Flow cytometric analysis of mesenchymal stem cells.

Mesenchymal stem cells (MSCs) from bone marrow (Lonza) were analyzed for expression of surface markers. MSCs expressed the known positive CD29 (Cat. No. 559883), CD44 (Cat. No. 555478), and CD90 (Cat. No. 555595) markers. Markers known to be negative on MSCs, CD34 (Cat. No. 555821) and CD45 (Cat. No. 555482), were not detected on these cells.

### Total solution kits for stem cell isolation and analysis

BD stem cell kits provide a streamlined solution for the reliable, high-purity isolation and in-depth characterization of stem cells by flow cytometry. These ready-to-use kits reduce experiment complexity and improve dependability by integrating many of the relevant reagents and methods required for cell sorting and analysis, including pre-titrated fluorochrome-conjugated antibodies to cell surface markers and transcription factors, compensation beads, buffers, protocols, and analysis guidelines. The BD Lyoplate™ screening panels are designed to help researchers quickly develop cell isolation and characterization strategies by providing direct quantitation of differentially-expressed markers.

High-quality monoclonal antibodies are key to consistent, reproducible results. Single clones are validated for multiple applications. Quality controls for high lot-to-lot consistency, protocol optimization, and validation are just a few ways that BD Biosciences reagents and assays help save time and enhance research productivity.

The BD Biosciences reagents collection is complemented by a rapidly expanding portfolio of fluorescently-labeled markers for a variety of stem cell types, in addition to multicolor reagents for new platforms such as immunofluorescent microscopy and bioimaging.

Today, cell surface markers are used to phenotype and isolate stem cells. In addition, directly-conjugated phospho-specific antibodies enable researchers to study cell signaling pathways of stem cells at the single-cell level.

## Services

For more than 25 years, BD has actively worked with stem cell researchers to develop tools that help improve workflow, ease of use, and performance. This in-depth knowledge and experience is available to customers through comprehensive training, application and technical support, and expert field service.

### Training

Held at BD training centers worldwide, BD Biosciences flow cytometry training courses combine theory and hands-on practice to provide participants with the skills and experience they need to take full advantage of the capabilities of their instrument.

### Technical Applications Support

BD Biosciences technical applications support specialists are available to provide field- or phone-based assistance and advice. Expert in a diverse array of topics, BD technical application specialists are well equipped to address customer needs in both instrument and application support.

### Field Service Engineers

BD Biosciences field service engineers are located across the world. When instrument installation or service is required, a BD Biosciences Technical Field Service Engineer can be dispatched to the customer site. On-site service and maintenance agreements are available to provide long-term support.

### Special Order Research Products

In addition to other services, BD instruments can be customized to meet specific customer requirements via the Special Order Research Products (SORP) program.

### Custom Services

Mobilizing technology for research applications requires close collaboration. The Custom Technology Team (CTT) at BD Biosciences works with customers to provide solutions through custom reagents, panels, or assay protocols.

Staffed by leading scientists with a breadth and depth of scientific and technical expertise, the CTT team will coordinate with researchers to study the problem at hand, make recommendations, and help implement solutions. In this way, BD Biosciences technical know-how is translated into practical solutions that allow customers to focus on research.

### BD BioCoat Custom Coating Service

The BD BioCoat Custom Coating Service offers an extensive selection of cell-based coatings on a wide variety of BD Falcon vessels, from roller bottles to flasks to 384-well plates. BD Biosciences can also develop special coatings. Customers specify the type of vessel and coating and BD can make a trial sample for evaluation.

### BD BioCoat Barcoding Service

The BD Biosciences Discovery Labware Barcoding Service provides high-quality barcode labels affixed to any side of a microplate. Barcodes have been quality tested for optimal readability, chemical resistance, and temperature durability.





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