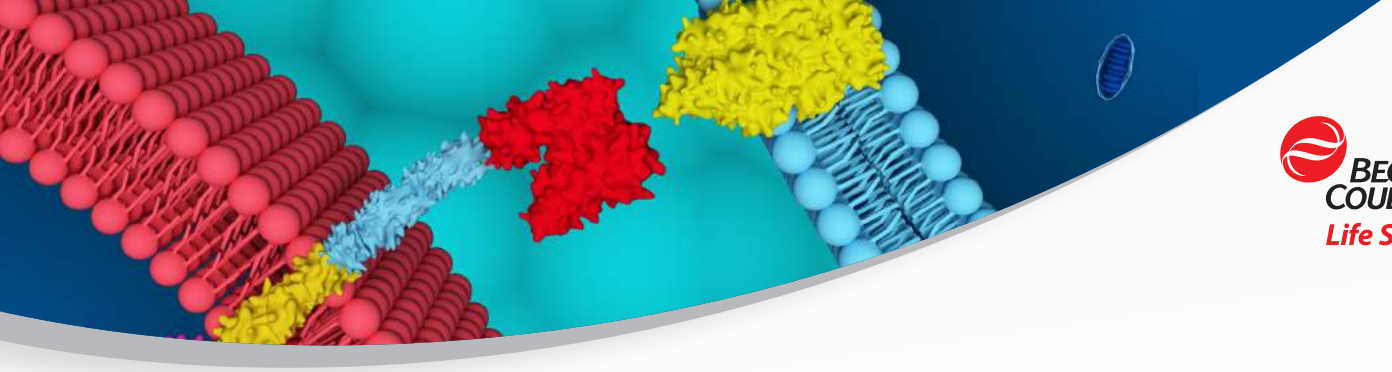
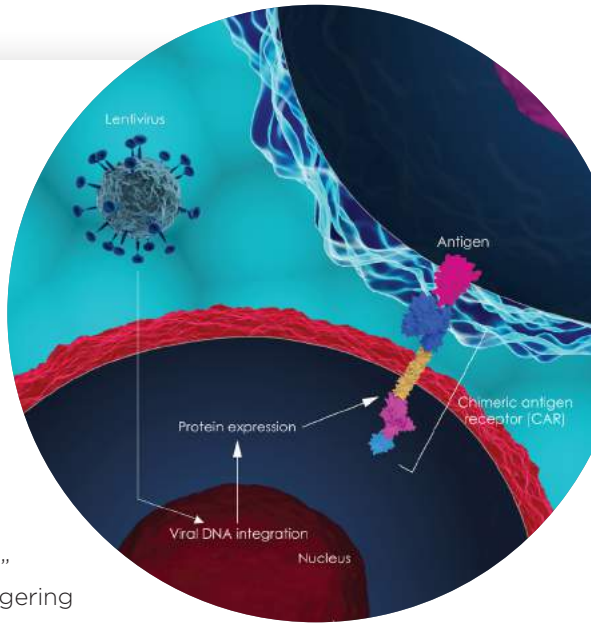


BECKMAN COULTER
Life Sciences

Manufacturing Processes for Engineered T-Cell Therapy – CAR-T

Why T cells are important

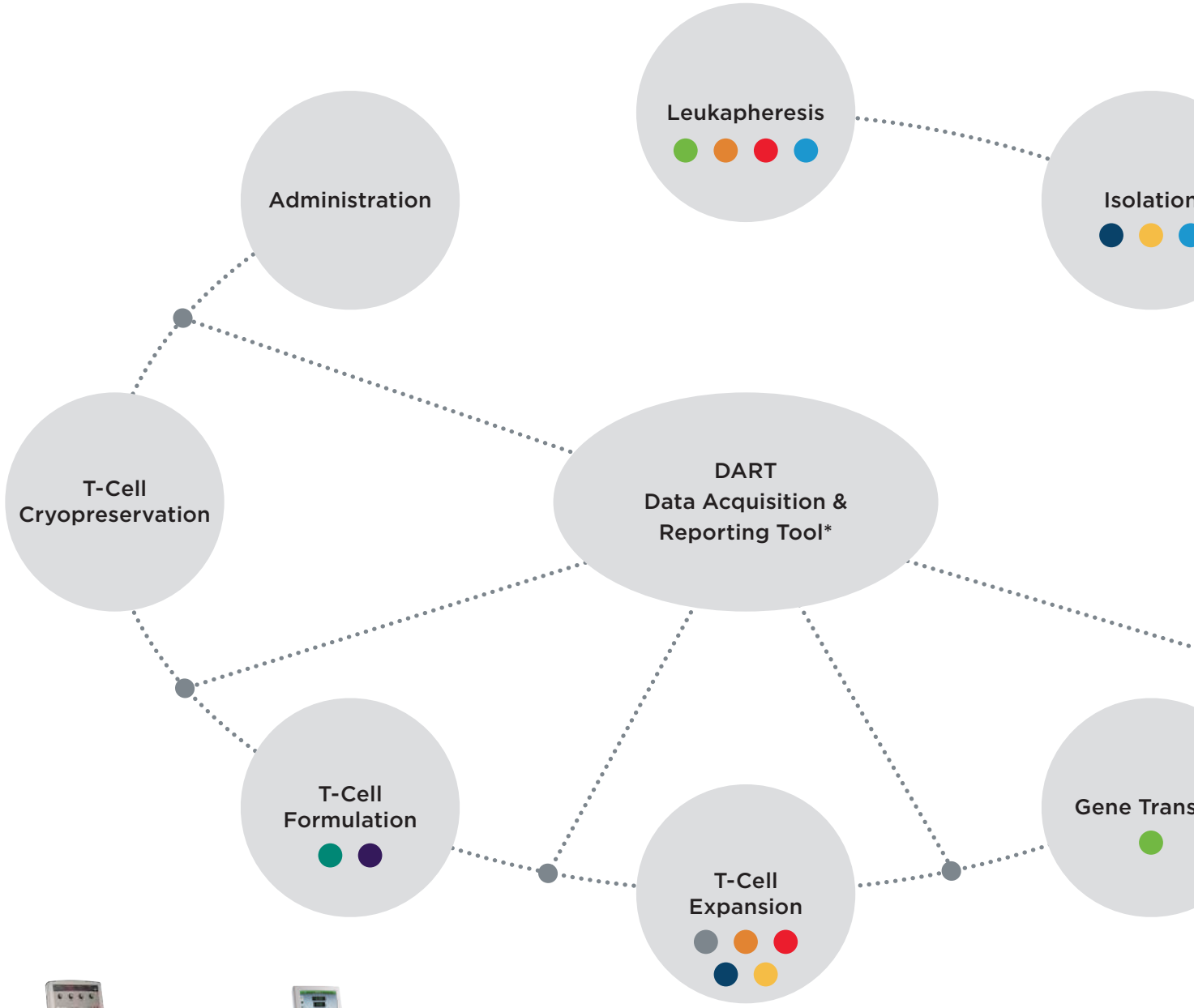
- They recognize host vs. non-host cells to initiate an immune response
- They're the primary immune system agents that attack diseased cells or infection
- They're used in several cell-based therapies:
  - **Transplantation of donor T-cells** - a bone marrow transplant, in which a matched donor's cells are used to replace the patient's cells, is the most widely used approach—typically for diseases that originate in the immune system (e.g., leukemia)
  - **Isolation and enrichment of a patient's T-cells** – an approach considered the safest because it enhances a patient's own immune response to disease
  - **CAR-T therapy** – a more recent approach in which T-cells are removed, genetically engineered to express a synthetic receptor against specific cancer cells, then infused back into the patient's bloodstream; this therapy is effective because many cancer cells "hide" from the immune system by appearing to be normal cells, thereby triggering no immune response



Examples of parameters collected during cell therapy production

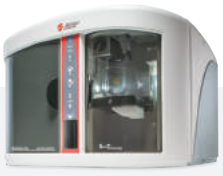
Data Collected	Why and When
Viable Cell Count	Used throughout process to monitor cell viability and impact of any cell manipulation events
Cell Size and Volume	Used as label-free method to monitor cell population during expansion, differentiation and/or transduction stages. Cell volume is a critical indicator of the activation state of the T-cells.
Cell Count	General population monitor, particularly during expansion stages and when sample volume is manipulated (e.g., during concentration and packaging)
Aggregate Detection	Primarily a concern during packaging and prior to administration
Gene Expression	Looking for specific surface marker expression or reporter gene expression
Metabolic Indicators	General monitoring during expansion stages when cells are under continuous culture
Immunophenotyping	Critical to ensure correct cell types are isolated and enriched in therapy population; often used to define purity

CAR-T Production Workflow



Avanti J-26S XP

Avanti JXN



Multisizer 4e²

Vi-CELL BLU²

Vi-CELL MetaFLEX²

*The DART data manager captures data from devices integrated into a Biomek robotic workstation. The Vi-CELL BLU and CytoFLEX can both be optionally integrated to a Biomek to enable this data capture and association with a sample ID.

Environmental Monitoring

MET ONE 3400

PAT700

Cancer Biopsy

Activation

T-Cell Transduction

Biomarker Identification

Generating the CAR Vector

Vector Production

CAR Engineering

CytoFLEX[1]

Biomek i5

Kaluza Software for Automated Data Analysis & LIS Connectivity[1]

DURA Innovations[1]

T-Cell Isolation

Output material generated in CAR-T cell manufacturing can be significantly affected by the starting raw materials. Leukapheresis is used to prepare/concentrate whole blood components (e.g., T-cells separated from red blood cells).

To maximize output of the CAR-T cell manufacturing process, samples are assessed for critical criteria, including:

- Cellular viability
- Change in cell volume
- Cell counts
- Rapid microbial testing
- Mycoplasma testing
- Immunophenotype

MULTISIZER 4e AND VI-CELL BLU ANALYZERS[2]

Defining conditions for cell viability and cellular proliferation are critical indicators of isolation conditions.

- Evaluate incoming samples
- Accurately analyze change in cell volume/size
- Rapidly determine cell count
- Supports 21 CFR Part 11 compliance

AVANTI J-26S XP HIGH-PERFORMANCE CENTRIFUGE FOR ELUTRIATION

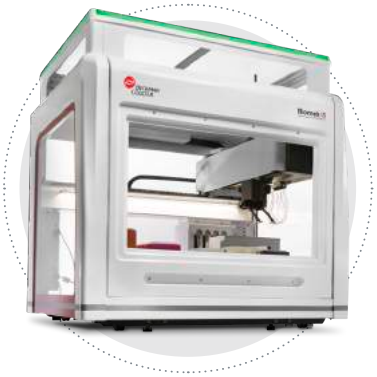
The counterflow centrifugation elutriation system is a gentle, yet powerful technique for harvesting large populations of living cells, resulting in high viability rates.

- Collects/separates cells using the JE-5.0 elutriation rotor
- Lets users select chamber flow rate and centrifugal speed based on cell size
- Uses counterflow to separate based on density/size
- Pushes cells into flask for easy, high-yield collection
- Uses centrifugal force versus flow pump

BIOMEK i5 LIQUID HANDLER

Dispensing raw material inputs into unitized formats reduces manual manipulation steps, thereby reducing variability. Biomek iSeries workstations further reduce operator-induced variability by shuttling samples to integrated end-point analysis platforms (e.g., cell counters, flow cytometers).

- Start-to-end automation
- Innovative integration options
- Supports 21 CFR Part 11 compliance



CYTOFLEX FLOW CYTOMETER[1]

Integrated CytoFLEX flow cytometers can phenotype and quality check raw materials for the manufacturing process to ensure standardization of protocols.

- Immunophenotyping of cells to characterize target T-cell populations
- Supports 21 CFR Part 11 compliance

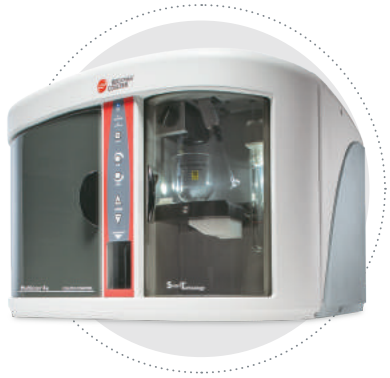
DURA INNOVATIONS[1]

Standardized dry antibody panels for comprehensive immunophenotypic characterization of source materials.

- Reduction of error-prone antibody pipetting
- Room temperature-stable unitized format, minimizing reagent inventory management
- Automation-friendly streamlined staining protocols
- GMP-compliant manufacturing

T-Cell Activation

T-cell activation and population expansion are critical for viral vector delivery and increased autologous dosage availability. Cell volume is a critical indicator of the activation state of the T-cell.



MULTISIZER 4e COULTER SIZE ANALYZER[2]

Monitor:

- Cell volume change after activation to determine cell state
- Cell proliferation during expansion

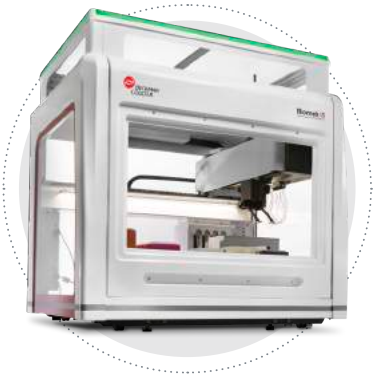
VI-CELL BLU AND VI-CELL METAFLEX ANALYZERS[2]

- Viable cell count using trypan blue if required/desired
- Rapid metabolite analysis to monitor bioreactor health

GENE TRANSFER: VIRAL VECTOR DELIVERY

Transformation of endogenous T-cell populations that carry a diverse array of antigen receptors into a homogeneous population with a target-specific receptor is the hallmark of CAR-T cell technology.

The process for efficient viral vector delivery presents significant challenges when performed manually. Viral vector delivery for CAR is achieved in small doses and by incremental up-scaling to improve transduction efficiency. Furthermore, culture conditions must be continuously monitored for in-process control.
For example the culture media is routinely assessed for bioanalytes such as pH, pO_2, pCO_2, glucose, lactate, electrolytes and more.



BIOMEK i5 LIQUID HANDLER

- Start-to-end automation
- Innovative integration options
- Supports 21 CFR Part 11 compliance

MULTISIZER 4e COULTER ANALYZER[2]

Monitor:

- Cell volume change after transdduction with CAR vector
- Cell proliferation during expansion

VI-CELL METAFLEX[2]

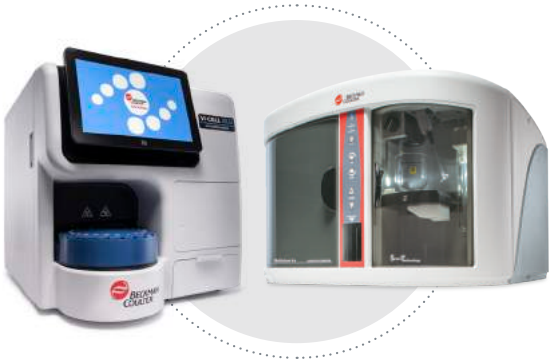
- Rapid in-process control
- Rapid metabolite analysis to monitor bioreactor health

T-Cell Expansion

MULTISIZER 4e AND VI-CELL BLU ANALYZERS[2]

Defining conditions for cell viability and cellular proliferation are critical indicators of isolation conditions.

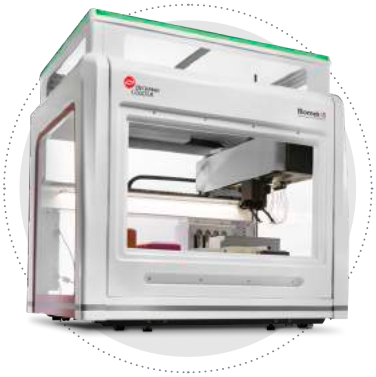
- Accurately analyze change in cell volume/size
- Rapidly determine cell count
- Supports 21 CFR Part 11 Compliance



BIOMEK i5 LIQUID HANDLER

Dispensing raw material inputs into unitized formats reduces manual manipulation steps, thereby reducing variability. Biomek iSeries workstations further reduce operator-induced variability by shuttling samples to integrated end-point analysis platforms (e.g., cell counters, flow cytometers).

- Start-to-end automation
- Innovative integration options
- Supports 21 CFR Part 11 compliance in regulated environments



CYTOFLEX FLOW CYTOMETER[1]

Integrated CytoFLEX flow cytometers can phenotype and quality check raw materials for the manufacturing process to ensure standardization of protocols.

- Immunophenotyping of cells to characterize target T-cell populations
- Supports 21 CFR Part 11 Compliance

DURA INNOVATIONS[1]

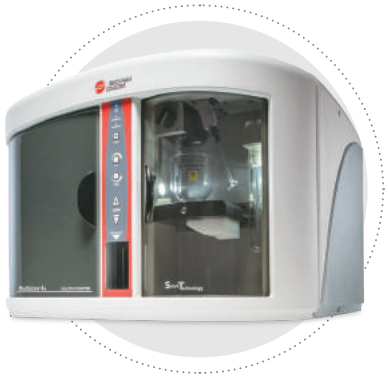
Standardized dry antibody panels and dry stimulation mixes for assessment of CAR T cell phenotype and function (e.g. IFNγ, TNFα, IL-2)cturing and expansion.

- Reduction of error-prone antibody pipetting and laborious handling of stimulation mixes
- Room temperature-stable unitized format, minimizing reagent inventory management
- Automation-friendly synchronous staining of surface markers and cytokines in a single step
- GMP-compliant manufacturing

T-Cell Formulation

Upon efficient manufacturing and expansion of CAR-T cells, characterization of output material is required for generating a standardized, unitized packaged product. Automated data collection and analysis reduce user-introduced bias for optimized formulation conditions.

MULTISIZER 4e AND VI-CELL BLU ANALYZERS[2]



Defining conditions for cell viability and cellular proliferation are critical indicators of isolation conditions.

- Evaluate finished product for change in cell volume and size analysis
- Rapidly determine cell count
- Maintain 21 CFR Part 11 compliance

CYTOFLEX FLOW CYTOMETER[1]

- Determine gene expression level of CAR vector
- Measure cell surface markers to confirm the appropriate cell population

KALUZA SOFTWARE WITH LIS CONNECTIVITY AND DART SOFTWARE[1]

Kaluza analysis software is designed to simply, efficiently and quickly analyze multicolor flow cytometry data. It enables you to:

- Easily transmit plots, statistics and keywords to a Laboratory Information System (LIS)
- Load any FCS-compliant file through standard 3.1
- Perform real-time processing of multicolor files of up to 20 million events
- Kaluza offers features supporting GMP compliance

DART (Data Acquisition and Reporting Tool) is a software package that gathers data and synthesizes runtime information from Biomek log files to capture each manipulation of the sample during the course of the method. DART enables you to:

- Browse live and historical run data from any device using a networked internet browser
- Reduce manual data integration
- Leverage historical data on labware for enhanced method programming
- Seamlessly port data between Biomek methods and individual liquid handlers
- Generate data reports via MS Excel table and pivot views, and SQL views
- Integrate with your Laboratory Information Management System

AVANTI HIGH-PERFORMANCE CENTRIFUGES

- Pellet cells from large harvest/culture
  - 1 L fixed-angle option (Avanti JXN-26 and JLA-8.1000)
  - 2.25 L swinging-bucket option (Avanti J-HC and JS-5.0)
- Package cells into HarvestLine bottle liners
  - Minimize risk of contamination
  - Obtain higher yields
  - Increase workflow efficiency

GMP Compliance Testing

ROUTINE ENVIRONMENTAL MONITORING FOR THE CLEANROOM ENVIRONMENT

- Industry's first embedded secure data transfer for supporting 21 CFR Part 11 compliance
- Cleanroom classification and monitoring to ISO 14644-1 & -2 as required by EU and USA GMP
- Well-executed environmental monitoring program test controls in place for maintaining cleanrooms to GMP standards
- Reduced complexity of cleanroom implementation/monitoring via automation of the data collection/reporting process

ON-LINE RELEASE TESTING FOR WFI AND PW

Total Organic Carbon and Conductivity Analyzer

- Specifically designed to help demonstrate compliance to the pharmacopoeial requirements for TOC and conductivity for purified water (PW) and water for injection (WFI)
- Secure electronic records for on-line release testing straight from the box

Why work with Beckman Coulter Life Sciences?

Our application experts have surveyed the market, looked at existing workflows and integrated our solutions at customer sites around the world. Let us help you minimize your R&D requirements by leveraging our experience and our workflow solutions.

- Our products are designed to support GMP compliance.**
- We offer PROService, an internet-based application that uses a proprietary data pipeline to remotely connect to our systems in your labs to help maximize instrument uptime and service efficiency.
- We're a Danaher company, with the Danaher Business System (DBS) at its core. DBS is a proven model for achieving success. It drives every aspect of our culture and performance. We use DBS to guide what we do, measure how well we execute, and create options for doing even better. Let us help you apply DBS to your lab.

Interested in talking to someone?

Visit **beckman.com/contact-us**

Learn more about CAR-T Solutions at
https://www.beckman.com/resources/research-areas

**The CytoFLEX, MET ONE 3400, PAT700, Multisizer 4e, Vi-CELL BLU, Vi-CELL MetaFLEX, and Kaluza are designed to support GMP compliance. Our Avanti JXN series centrifuges have enhanced data management which provides the capabilities to support customers' GMP processes

1 For research use only. Not for diagnostic purposes. All other products identified are not for use in diagnostic procedures.

2 The Multisizer 4e, Vi-CELL BLU, and Vi-CELL MetaFLEX are for Laboratory Use Only.

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