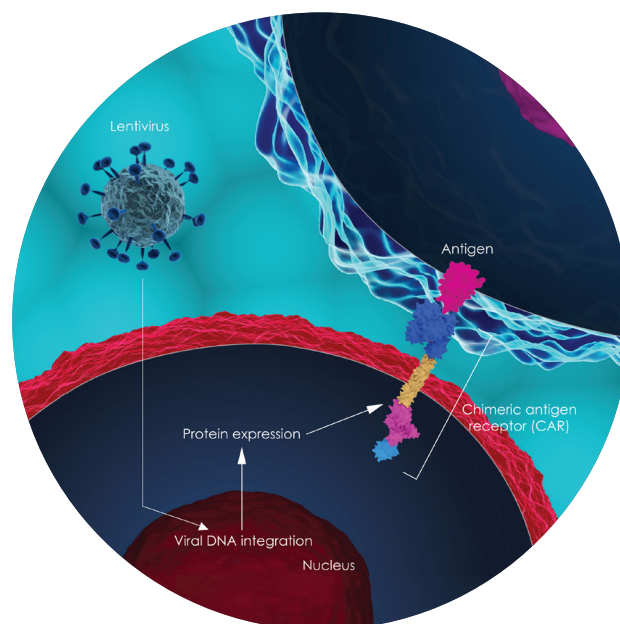


Manufacturing Processes for Engineered T-Cell Therapy – CAR-T

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



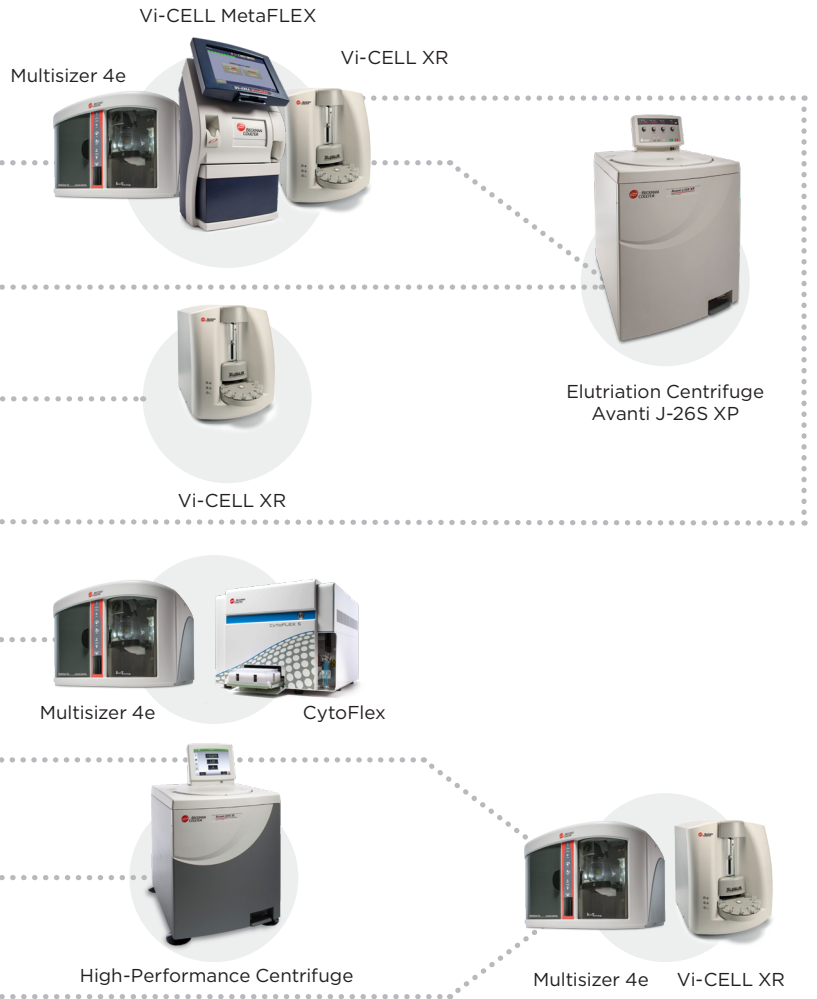
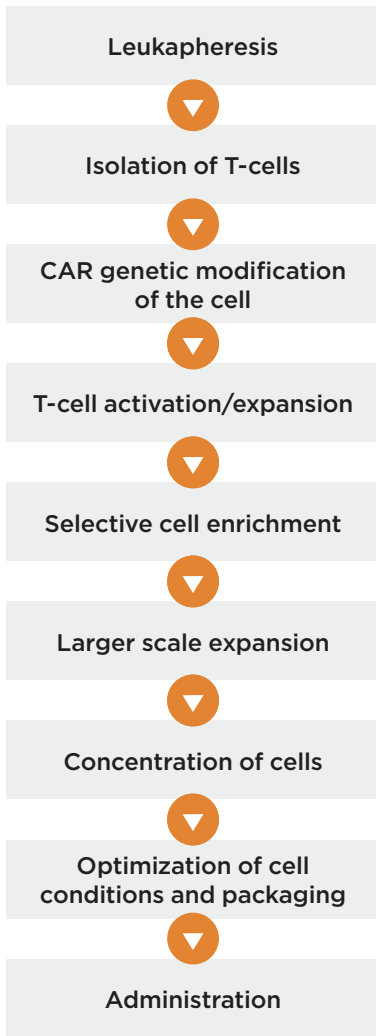
Examples of parameters collected during cell therapy production

Data Collected	Why and When
Viable Cell Count	Used throughout process to monitor cell health 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 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 patient 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

Learn more about CAR-T Solutions at Beckman.com/home

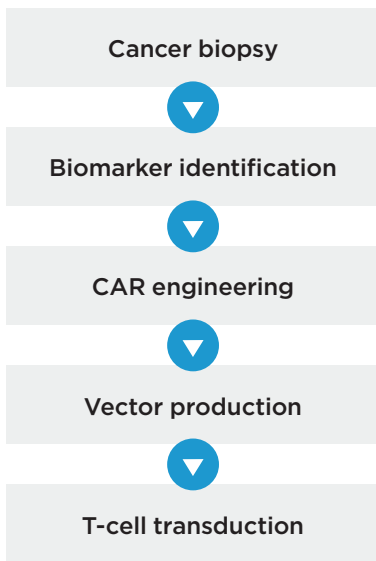
CAR-T production workflow

T-cell Handling Workflow



Cryopreservation (Optional)

Generating the CAR Vector



Leukapheresis, sample screening and viability monitoring

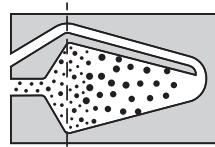
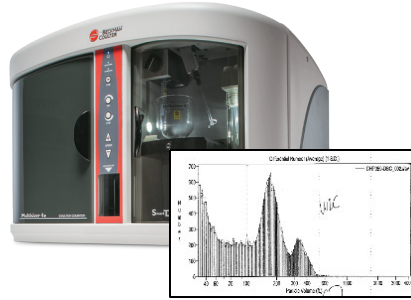
Multisizer 4e Coulter Analyzer

- Evaluate incoming samples
- Accurate cell volume and size analysis
- Rapid cell count

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.

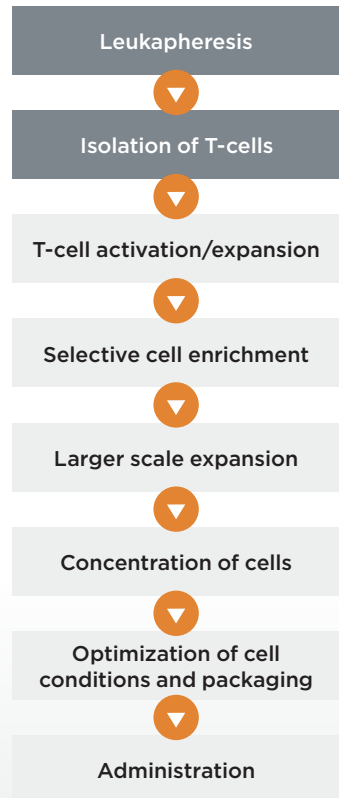
- Collection and separation of cells using the JE-5.0 elutriation rotor
- Select chamber flow rate and centrifugal speed based on cell size
- Uses counterflow to separate based on density and size
 - Centrifugal force versus flow pump
 - Pushes cells into flask for easy, high-yield collection



Elutriation Boundary



T-cell activation and population expansion



T-cell activation and population expansion

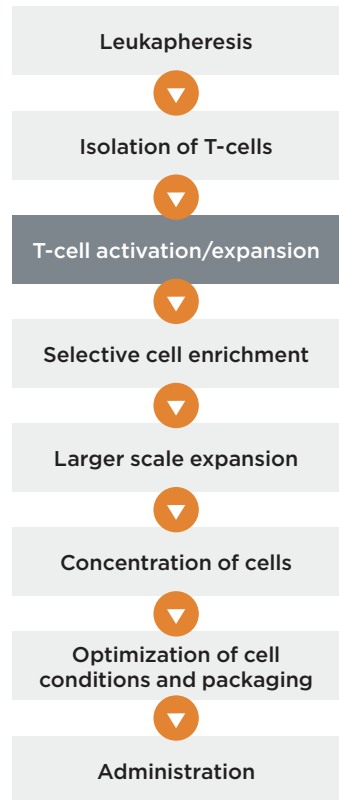
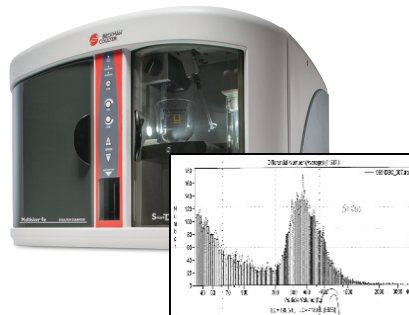
Cell volume is a critical indicator of the activation state of the T-cell.

Multisizer 4e Coulter Analyzer

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

Vi-CELL XR and MetaFLEX

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



T-cell enrichment and characterization

Multisizer 4e Coulter Analyzer

- Monitor cell volume change after transduction with CAR vector
- Monitor cell proliferation during expansion

CytoFLEX Flow Cytometer

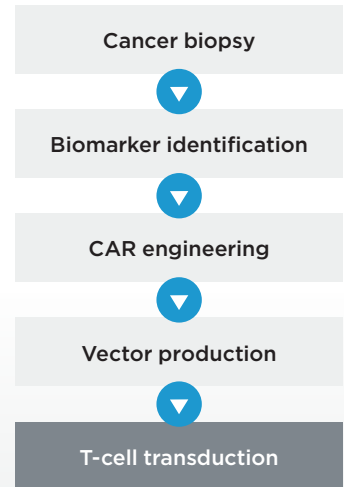
- Determine gene expression level of CAR vector
- Measurement of cell surface markers to confirm the appropriate cell population

Vi-CELL XR and MetaFLEX

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



Generating the CAR Vector



Collection, concentration, packaging and QC of T-cells

Multisizer 4e Coulter Analyzer

- Evaluate incoming samples
- Accurate cell volume and size analysis
- Rapid cell count

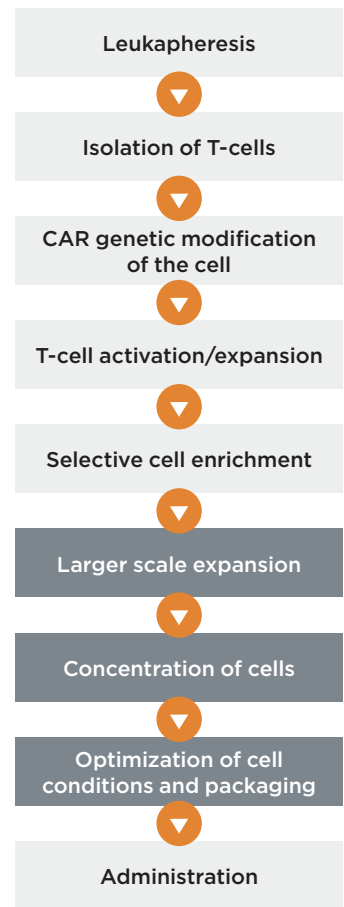
Avanti High-Performance Centrifuges

Pelleting of cells from large harvest/culture

- 1L fixed-angle option with the Avanti JXN-26 and JLA-8.1000
- 2.25L swinging bucket option with the Avanti J-HC and JS-5.0

Packaging of cells into Harvest Liner System

- Minimize risk of contamination
- Higher yields
- Increase in workflow efficiency



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