NEO GENOMICS

Flow Cytometry

Your global partner for
flow cytometry services supporting clinical development programs

Rely on our flow cytometry expertise and capabilities to deliver on your project

- **From discovery through clinical phases**
- **Across therapeutic disciplines and applications**
- **Global, with all sites CAP-accredited**

Highlights of our flow cytometry program

Custom assay design, development, and validation to support programs at any phase

Technical and medical expertise across applications, including hematopoietic neoplasms, MRD, pharmacodynamics, infectious disease and immuno-oncology

Exploratory to clinically validated panels and a large menu of validated antibodies for the optimal fit-for-purpose assay option

Global harmonization with rigorous quality standards supports global trials and recruitment

Custom assay design, development, and validation expertise support your specific program objectives

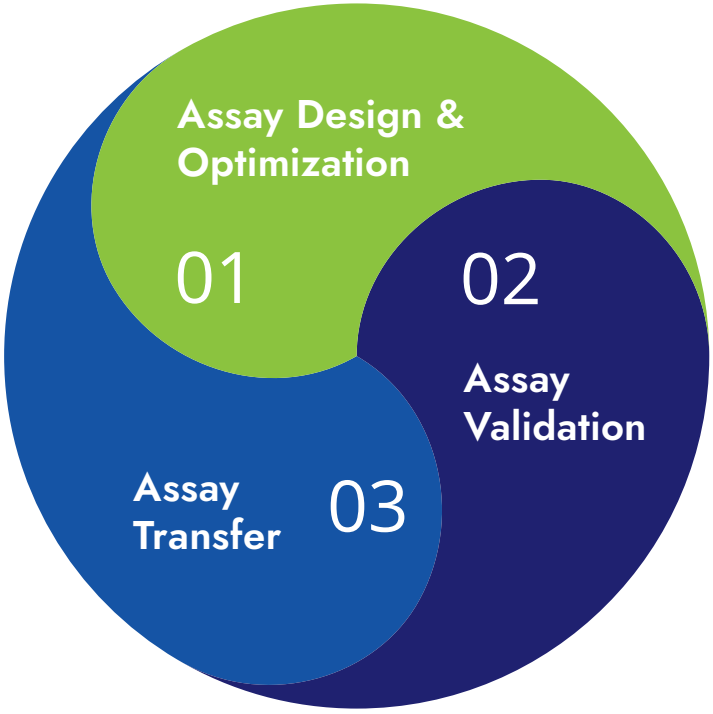
We work closely with our partners from the beginning to better understand program objectives and develop a plan that supports meeting them. Our business, scientific, medical, and project management teams have ongoing consultations to ensure gaps in the project plan are addressed and that the final assay design will generate the required data.

Depending on specific project needs, we support different assay development options. Scenarios routinely support the range from developing and validating fully custom fit-for-purpose assays, building assays from our library of existing clinically validated modules and antibodies, or transferring an assay from a sponsor. Whatever your choice, our highly experienced teams deliver.

To ensure the highest quality assay possible, we maintain a high set of standards, including harmonized instrument calibration and specific reagent qualification for consistent assay performance globally. Other formal processes for assay transfer provide a robust and controlled transfer with data reproducibility at all global testing locations.

As programs evolve, we do too. When an exploratory assay identifies clinically valuable markers, we can further develop the assay and validate it for clinical use.

Highly efficient and thorough process for flow assay development



01 Assay design/optimization

- Establish assay requirements
- Assay design and reagent qualification
- Instrument qualification

02 Assay validation

- Precision, reproducibility, accuracy
- Sample storage / long-term stability
- Robustness / LOD / LOQ
- Specificity, reference sample, QC

03 Operationalization

- Assay implementation
- Transfer
- Harmonization

Technical and medical expertise across disciplines supports drug development, vaccine development and research across disease areas

Our scientific and medical teams are experienced in flow cytometry assessments supporting many applications, including hematopoietic neoplasms, MRD, pharmacodynamics, infectious disease, and immuno-oncology. We also offer other supporting services like harmonized and global PBMC processing for retrospective or functional testing.

Examples of supported complex assessments used in drug and vaccine development include immunophenotyping of subsets, MDSC/dendritic, activation/exhaustion, rare event, receptor occupancy, cell signaling, cell function, and checkpoint molecules. For LDT development and validation, we support applications including MRD status, malignant cell characterization, CAR-T detection with custom anti-CAR-T antibody, and TBNK enumeration.

With up to 30-color analysis capability utilizing the Cytek Aurora, 16-color analysis utilizing the BD LSR Fortessa X-20 instrument or 10-color Beckman Coulter Navios clinical diagnostic instrumentation, a vast array of capabilities are available. From complex multi-color immunophenotyping to minimal residual disease analysis, NeoGenomics Pharma Services has the means and skill to suit your needs.

Custom assay development by application

Hematopoietic Neoplasms	**Leukemia / Lymphoma**	CD2, CD3, CD4, CD5, CD7, CD10, CD11b, CD11c, CD13, CD14, CD15, CD16, CD19, CD20, CD22, CD23, CD33, CD34, CD38, CD41, CD45, CD56, CD64, CD71, CD117, CD138, HLA-DR, TdT, MPO
		CD5, CD10, CD11c, CD19, CD20, CD22, CD23, CD34, CD43, CD45, CD3b, CD103, CD200, Kappa, Lambda, FMC-7
		CD1a, CD2, CD3, CD4, CD5, CD7, CD8, CD19, CD33, CD45, CD56
	Multiple Myeloma	CD19, CD20, CD38, CD45, CD56, CD117, CD138, Kappa, Lambda
	PNH	CD14, CD15, CD24, CD25, CD45, CD59, CD64, 235a, FLAER
	Diagnosis/Prognosis	Biomarker panels in MM, CLL, AML, ALL, lymphoma and prognostic markers
	Minimal Residual Disease	MRD panels for B-ALL, MM, and CLL
Pharmacodynamics (PD)	**Custom Receptor Occupancy Assays**	Determination of biological effective dose of target expression and engagement
Immuno-Oncology	**T-Cell Profiling**	Activation and Exhaustion, Immunophenotyping, Differentiation
		Enumeration of T-cell subsets: Th1, Th2, Th17, T-reg and CAR-T Cell Function / Cell Signaling
	B-Cell Phenotyping	Comprehensive B-cell differentiation
	MDSC / Dendritic Cells	mMDSC, gMDSC, mDC, pDC

Supporting all phases of development from exploratory panels to clinically validated LDTs with primary and/or secondary endpoints

During the pre-initiation phase, we consult with our partners to fully understand the intended utility of the assay. Getting this right at the beginning facilitates project success as it drives many downstream decisions.

We design, develop, and validate assays that are fit-for-purpose from early phase RUO/exploratory assays to later phase clinically validated LDTs with primary and/or secondary endpoints. As projects move across phases, we have the expertise and capabilities to execute, including moving exploratory assays into the LDT space.

Decision making for flow assay design and validation

Client Request

Determination of Regulatory Requirements and Intended Use

CLIA/LDT

- Primary/Secondary Endpoint, Patient Stratification, Enrollment
- Patient Treatment, Safety CDx
- Full Pathology Review/ Interpretation and Data Release

- MRD Status
- Malignant Cell Characterization
- CAR-T Detection
- TBNK Enumeration

RUO/EXPLORATORY

- Biomarker Evaluation, Non-safety, No Inclusion/Exclusion
- Exploratory Endpoint, Drug Targeted
- Research Use Only Data

- Immunophenotyping (Subsets, MDSC/Dendritic, Activation/ Exhaustion, Rare Event, etc.)
- Receptor Occupancy
- Cell Signaling/Cell Function/Assays
- Apoptosis/Checkpoint Molecules

LDT flow assay example

MM MRD

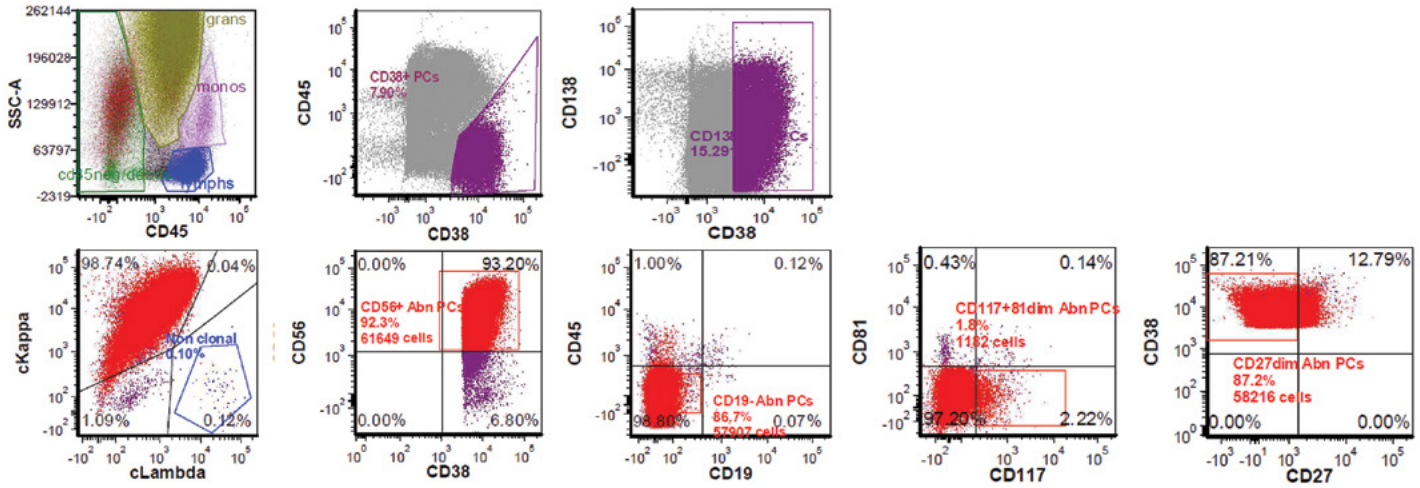
Assay development requirements

- Access MM patient bone marrow
- 0.001% sensitivity

Assay parameters

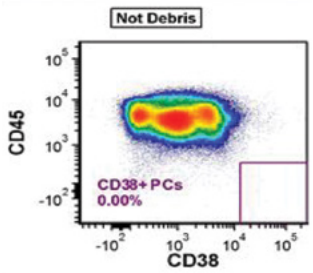
- 3-5 million events collected
- 10 markers
- Single tube assay (10 color)

Fluorochrome	FITC	PE	PC 5.5	PE-Cy7	BV 421	BV510	BV 605	APC	APC-A700	APC-H7
MM MRD	**cKappa**	**cLambda**	**CD117**	**CD19**	**CD81**	**CD38**	**CD27**	**CD138**	**CD56**	**CD45**



LDT flow assay example — outputs

- Output includes Diagnosis and Interpretation
- Pathologist Review and Signoff
- Reported to clinical site investigator for potential patient treatment decisions in addition to sponsor



Diagnosis:
- Clonal plasma cells are identified:
%MRD of total nucleated cells: 0.002%
MRD count: 70

Percentages from CD38+ and CD138+ gate (70 events)
CD56: 45 events, 64.29%
CD117: 2 events, 2.86%
CD81: 12 events, 17.14%
CD27: 4 events, 5.71%
CD19: 13 events, 18.57%
Non Clonal: 2 events, 2.86%

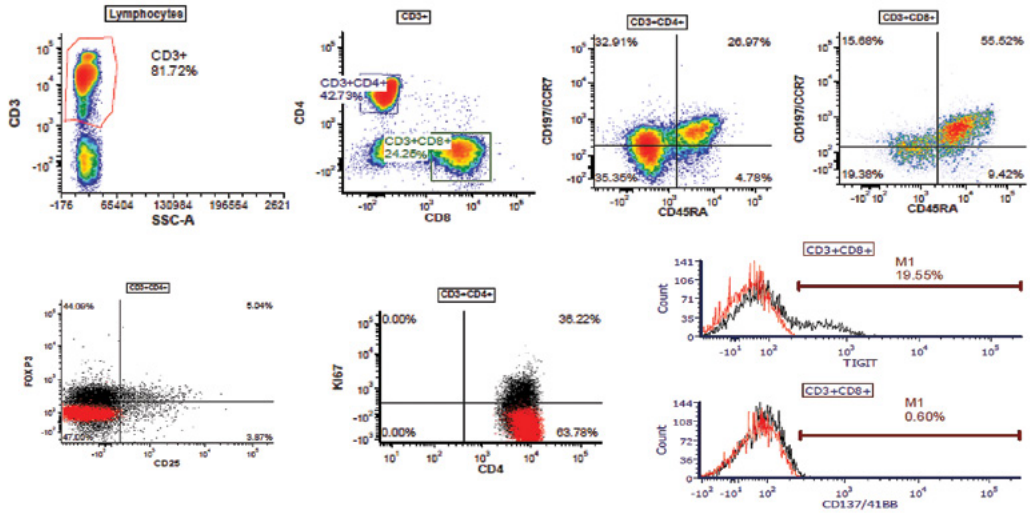
Markers Performed:
CD19, CD27, CD38, CD45, CD56, CD81, CD117, CD138, cKappa, cLambda (10 Markers)

Electronic Signature

Exploratory/RUO flow assay example — outputs

- Output are data points / raw data
- No pathology review required
- Data is reported to sponsor or third party
- Results are for research use only and no patient treatment decisions can be made

Lasers	Blue					Red			Violet						Ultraviolet	
Fluoro-chrome	FITC	PE	PE Dazzle 594	PE-Cy7	PerCP-Cy5.5	AF 647	APC-R700	APC-Fire 750	BV 421	BV 510	BV 605	BV 650	BV 711	BV 785	BUV 395	BUV 525
T-Cell Profiling Assay	CD4	Blank	Blank	Blank		Blank	CD3	Blank	Blank	CD45 RA	CCR7 (CD 197)		Blank	Blank	CD8	
	CD4	CD137 (4-1BB)	TIGIT	PD-L1 (CD 274)		Ki-67	CD3	PD-1 (CD 279)	FoxP3	CD45 RA	CCR7 (CD 197)		CD25	LAG3 (CD 223)	CD8	



16 Color Flow Capability Ideal for Exploratory Panel Development

Lymphocytes	9.48%	CD3+CD4+PD-1+	0.00%	CD3+CD4+PD-L1+	2.89%
CD3+CD4+CD137 (4-1BB)+	0.15%	CD3+CD4+LAG3+	0.86%	CD3+CD4+Ki67+	12.92%
CD3+CD4+TIGIT+	5.45%	CD3+CD4+CD25+	7.47%	CD3+CD4+CD25+FoxP3+PD-1+	0.00%
CD3+CD4+CD25+FoxP3+PD-L1+	4.55%	CD3+CD4+CD25+FoxP3+CD137+	2.27%	T-cells CD3+	71.38%
CD3+CD4+CD25+FoxP3+LAG3+	0.00%	CD3+CD4+CD25+FoxP3+TIGIT+	15.15%	CD3+CD4+CD25+FoxP3+Ki67+	22.73%
CD3+CD8+CCR7+CD45RA+	15.06%	CD3+CD8+CCR7+CD45RA-	0.37%	CD3+CD8+CCR7-CD45RA-	29.04%
CD3+CD8+CCR7-CD45RA+	55.53%	CD3+CD8+PD-1+	0.00%	CD3+CD8+PD-L1+	4.64%
CD3+CD8+CD137 (4-1BB)+	0.00%	Helper T-cells CD3+CD4+	42.59%	CD3+CD8+LAG3+	0.00%
CD3+CD8+Ki67+	15.41%	CD3+CD8+TIGIT+	8.58%	CD3+CD8+CD25+	0.48%
Cytotoxic T-cells CD3+CD8+	25.11%	CD3+CD4+CD25+FoxP3+ Treg	0.73%	CD3+CD4+CCR7+CD45RA+	5.64%
CD3+CD4+CCR7+CD45RA-	2.27%	CD3+CD4+CCR7-CD45RA-	67.47%	CD3+CD4+CCR7-CD45RA+	24.62%

Run trials and recruit around the globe with harmonized global capabilities

Patient recruitment has become more challenging with the steady rise in the number of trials. Meet your recruitment targets with flow capabilities in the U.S. and Europe. Multiple locations provide clear advantages but can also pose challenges for data quality. Our robust harmonization protocols for instruments, reagents, and assays eliminate issues like poor data reproducibility and high variation between sites. Our global sites also have other major modalities, making them well suited as primary sites for trials requiring additional modalities.

To learn more about NeoGenomics Pharma Services, visit us online at neogenomics.com/pharma-services, call us at 800.720.4363 or email us at pharmaservices@neogenomics.com

NeoGenomics Laboratories is a specialized oncology reference laboratory providing the latest technologies, testing, partnership opportunities, and interactive education to the oncology and pathology communities. We offer the complete spectrum of diagnostic services in molecular testing, FISH, cytogenetics, flow cytometry, and immunohistochemistry through our worldwide network of CAP-accredited, CLIA-certified laboratories.

Committed to research as the means to improve patient care, we provide Pharma Services for pharmaceutical companies, in vitro diagnostic manufacturers, and academic scientist-clinicians. We promote joint publications with our client physicians. NeoGenomics welcomes your inquiries for collaborations. Please contact us for more information.

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