**The laboratory ensures that immunoglobulin staining is intrinsic and not extrinsic (cytophilic).**

*NOTE: The requirement is to ensure that the immunoglobulin light chain analysis includes only light chain synthesized by B cells (intrinsic light chain). Many cell types will bind serum immunoglobulin nonspecifically via Fc receptors (including B cells). To ensure that immunoglobulin staining detected by flow cytometry is intrinsic (on B cells) rather than cytophilic, a pan-B cell marker (eg, CD19, CD20) may be included in the same tube as one or both anti-light chain reagents. The inclusion of both lambda and kappa light chain reagents in the same tube allows a clear delineation of non-specific binding, even on B cells.*

REFERENCES
1) Clinical and Laboratory Standards Institute (CLSI). *Clinical Flow Cytometric Analysis of Neoplastic Hematolymphoid Cells; Approved Guideline—Second Edition*. CLSI document H43-A2. Clinical and Laboratory Standards Institute, Wayne, PA; 2007.

## FLO.30730 Abnormal Cell Distinction — Phase II

**Abnormal cells of interest are appropriately distinguished from normal cells based on their light scatter and fluorescence properties.**

*NOTE: Generally, both neoplastic and non-neoplastic cells are acquired in any gate used for acquisition. Attempts must be made to distinguish them at the time of analysis. Appropriate procedures include use fluorescent antibodies, fluorescent dyes, light scatter measurements, or any combination thereof to select out the relevant cell subpopulation for further analysis. Morphologic evaluation is also a valuable parameter to improve analysis.*

*Laboratories performing interpretation only of immunophenotyping data from an outside facility (ie, technical flow laboratory) must ensure that appropriate gating techniques are used. There must be a process by which individuals interpreting the results can provide feedback on the appropriateness of the gating techniques used. Records of such feedback and corrective action taken when problems are identified may be incorporated into the laboratory's quality management system.*

REFERENCES
1) Muirhead KA, *et al*. Methodological considerations for implementation of lymphocyte subset analysis in a clinical reference laboratory. *Ann NY Acad Sci.* 1986;468:113-127
2) American Society for Microbiology. Manual of clinical immunology, 4th ed. Washington, DC: ASM, 1992
3) Sun T, *et al*. Gating strategy for immunophenotyping of leukemia and lymphoma. *Am J Clin Pathol.* 1997;108:152-157
4) Clinical and Laboratory Standards Institute (CLSI). *Clinical Flow Cytometric Analysis of Neoplastic Hematolymphoid Cells; Approved Guideline—Second Edition*. CLSI document H43-A2. Clinical and Laboratory Standards Institute, Wayne, PA; 2007.
5) Macon WR, Salhany KE. T-cell subset analysis of peripheral T-cell lymphomas by paraffin section immunohistology and correlation of CD4/CD8 results with flow cytometry. *Am J Clin Pathol.* 1998;109:610-617
6) Dunphy CH. Combining morphology and flow cytometric immunophenotyping to evaluate bone marrow specimens for B-cell malignant neoplasms. *Am J Clin Pathol.* 1998;109:625-630
7) Clinical and Laboratory Standards Institute (CLSI). *Validation of Assays Performed by Flow Cytometry - Approved Guideline-First Edition.* CLSI Document H62. Clinical and Laboratory Standards Institute, Wayne, PA, 2021.

## FLO.30760 Cell Population Distinction — Phase II

**The laboratory has a defined process to distinguish fluorescence-negative and fluorescence-positive cell populations.**

*NOTE: This does not imply that a separate negative control sample must be run. It is possible to coordinate panels of monoclonal antibodies to compare the binding of monoclonal antibodies of the same subclass that typically have mutually exclusive patterns of reactivity of subsets of hematopoietic cells. In this way, test antibodies may also double as control reagents.*

REFERENCES
1) Clinical and Laboratory Standards Institute (CLSI). *Clinical Flow Cytometric Analysis of Neoplastic Hematolymphoid Cells; Approved Guideline—Second Edition*. CLSI document H43-A2. Clinical and Laboratory Standards Institute, Wayne, PA; 2007.
2) Clinical and Laboratory Standards Institute (CLSI). *Validation of Assays Performed by Flow Cytometry - Approved Guideline-First Edition.* CLSI Document H62. Clinical and Laboratory Standards Institute, Wayne, PA, 2021.