Kappa and Lambda Reagents

Flow Cytometry

Dako

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Kappa and Lambda Reagents for Flow Cytometry

Kappa and lambda reagents are important in the diagnosis and identification of hematopoietic malignancies of B-cell origin. Such malignant cells are clonal in nature typically expressing either kappa or lambda on their cell surface and cytoplasmically. Thus, analysis of suspicious cell populations using reagents specific for either kappa or lambda can be used to demonstrate clonality (1), which is a hallmark of malignancy.

Several B cell lymphoproliferative disorders and plasma cell dycrasias are associated with decreased levels of kappa and lambda at the cell surface. In these cases, it is important to utilize kappa and lambda reagents that can optimally identify kappa and lambda cell surface expression even at low levels.

In some B-cell malignancies the kappa and lambda light chains may be mutationally altered creating a risk that the use of monoclonal antibodies for analysis could fail to detect these light chains. Using polyclonal antibodies that recognize multiple epitopes on the target molecule eliminates this risk and helps avoid false-negative results (2). Furthermore, the binding of more than one reagent per target molecule will increase the staining intensity.

Advantages of using polyclonal antibodies in the detection of immunoglobulin light chains

A study (3) has demonstrated the advantage of using polyclonal antibodies in the clonality assessment of plasma cells. Bone marrow samples of a myeloma patient were analyzed for the presence of residual abnormal plasma cells by multicolor flow cytometry. Absence of cytoplasmic light chain expression in a plasma cell subpopulation with an abnormal phenotype using monoclonal antibodies suggested the presence of non-secretory plasma cells in the bone marrow of this patient (Figure A). This observation however, was contradicted by the presence of free lambda light chains in the patient's serum. After repeating the analysis with polyclonal antibodies against intracellular immunoglobulin light chains instead of monoclonal antibodies, the abnormal plasma cell subpopulations were shown to express lambda light chains across all tested subpopulations (Figure B). The data from the study clearly demonstrate that usage of polyclonal over monoclonal antibodies should be preferred for the detection of intracellular immunoglobulin light chains.

Another study (4) illustrates the occurrence of false-negative results when using a monoclonal anti-light chain antibody in the assessment of clonal B cells in a patient with follicular lymphoma. In the study, the analysis for surface immunoglobulin light chains (SIg) using monoclonal antibodies failed to detect surface kappa and lambda light chains in one of the assessed CD20+ subpopulations (Figure C). On repeating the tests using polyclonal antibodies, both the cell populations were observed to be positive for surface kappa and lambda light chains (Figure D). This suggested that the epitope recognized by the monoclonal antibody is not expressed or accessible in all B-cell populations leading to false-negative results. The study advocates the preference and use of polyclonal antibodies in the detection of membrane-bound immunoglobulin light chains.

Figure A: Plasma cells in subpopulation II do not stain for cytoplasmic kappa and lambda light chains using monoclonal antibodies.

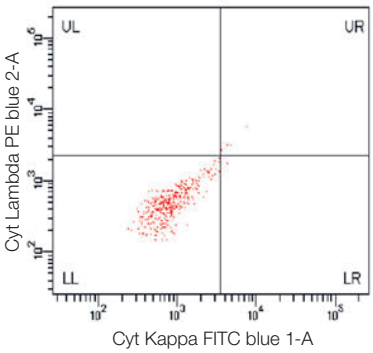


Figure B: Plasma cells in subpopulation II stain for cytoplasmic lambda light chains using polyclonal antibodies.

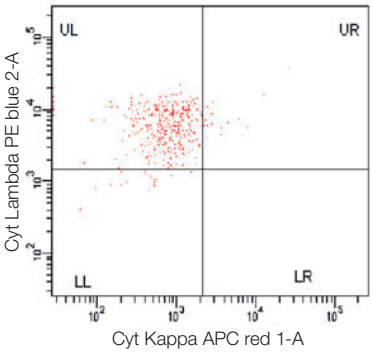


Figure C: Subpopulation II does not stain for surface kappa and lambda light chains using monoclonal antibodies.

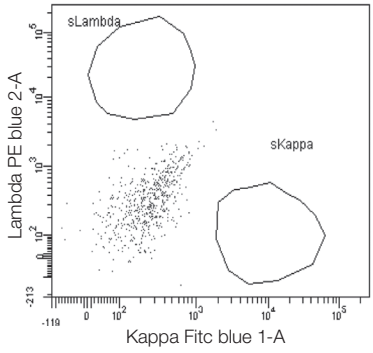
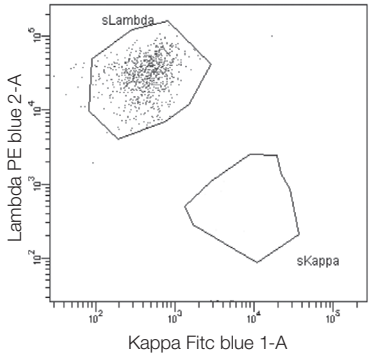


Figure D: Subpopulation II is positive for surface lambda light chains when stained with polyclonal antibodies.



Advantages of Dako's polyclonal kappa and lambda reagents

- All of Dako's kappa and lambda reagents are designated for use in the clinical diagnostics laboratory for clinical applications.
- The performance of polyclonal antibodies is not susceptible to mutational changes in the epitope.
- Polyclonal antibodies bind to multiple epitopes while monoclonal antibodies bind to only one epitope. Many polyclonal antibodies conjugated with fluorescent chromogens render brighter signals than equivalent monoclonal antibodies.
- The use of F(ab')$_2$-fragmented antibodies mitigates non-specific binding by eliminating Fc-mediated binding to non-target cells. Dako's kappa and lambda reagents are not whole Ig molecules but instead F(ab')$_2$ fragments.
- Dako's polyclonal kappa and lambda antibodies mitigate lot-to-lot performance difference as each single reagent lot comes from an extensive pool of antibodies.
- With more than 45 years of experience within antibody production, Dako offers reagents of excellent quality.

"We have used the MultiMix™ Triple-Color Reagent, Anti-Human CD19/FITC + Anti-Human Lambda Light Chains/RPE + Anti-Human Kappa Light Chains/APC for the last year and **have found it excellent for enumeration of kappa and lambda expressing B cells.**

This is particularly important in the investigation of some lymphoproliferative disorders such as CLL where the expresssion of kappa or lambda may be very weak.

The choice of fluorochromes for kappa and lambda is ideal as it utilizes bright fluorochromes and hence provides better staining than some other commercial reagents. We have also found the premixed combination convenient to use in our laboratory."

Charles Pearson, Haematology, Glasgow Royal Infirmary

ORDERING INFORMATION

Product	Size	Code
Single-Color Reagents		
Polyclonal Rabbit Anti-Human Kappa Light Chains/APC, Rabbit F(ab')$_2$	100 tests, 1 mL	C0222
Polyclonal Rabbit Anti-Human Kappa Light Chains/FITC, Rabbit F(ab')$_2$	100 tests, 1 mL	F0434
Polyclonal Rabbit Anti-Human Kappa Light Chains/RPE, Rabbit F(ab')$_2$	100 tests, 1 mL	R0436
Polyclonal Rabbit Anti-Human Lambda Light Chains/FITC, Rabbit F(ab')$_2$	100 tests, 1 mL	F0435
Polyclonal Rabbit Anti-Human Lambda Light Chains/RPE, Rabbit F(ab')$_2$	100 tests, 1 mL	R0437
MultiMix™ Dual-Color Reagents		
MultiMix™ Dual-Color Reagent, Anti-Human Lambda Light Chains/FITC + Anti-Human CD19/RPE	50 tests, 0.5 mL	FR044
MultiMix™ Dual-Color Reagent, Anti-Human Kappa Light Chains/FITC + Anti-Human CD19/RPE	50 tests, 0.5 mL	FR048
MultiMix™ Dual-Color Reagent, Anti-Human Kappa Light Chains/FITC + Anti-Human Lambda Light Chains/RPE	50 tests, 0.5 mL	FR481
MultiMix™ Triple-Color Reagents		
MultiMix™ Triple-Color Reagent, Anti-Human Kappa Light Chains/FITC + Anti-Human Lambda Light Chains/RPE + Anti-Human CD19/RPE-Cy5	50 tests, 0.5 mL	TC051
MultiMix™ Triple-Color Reagent, Anti-Human Plasma Cell/FITC + Anti-Human Lambda Light Chains/RPE + Anti-Human Kappa Light Chains/APC	50 tests, 1 mL	TC670
MultiMix™ Triple-Color Reagent, Anti-Human CD19/FITC + Anti-Human Lambda Light Chains/RPE + Anti-Human Kappa Light Chains/APC	50 tests, 1 mL	TC669

EU regulatory status: CE-IVD

"**The Dako kappa/lambda products are so bright** that they have significantly reduced the number of times we have to perform intracellular Ig analysis to confirm the presence of restricted B-cell populations"

Jo-Anne Lemenager, Clinical Laboratory, ConVerge Diagnostic Services

References

1. Nguyen D, Diamond LW, Brayland RC. Flow cytometry in haematopathology. Humana Press Inc 2003:77-9.
2. Nguyen D, Diamond LW, Brayland RC. Flow cytometry in haematopathology. Humana Press Inc 2003:18-9.
3. van Velzen JF, van den Blink D, Bloem AC. Inability of a monoclonal anti-light chain antibody to detect clonal plasma cells in a patient with multiple myeloma by multicolor flow cytometry. Cytometry Part B 2013;84B:30-2.
4. van Velzen JF, van den Blink D, Wiegers IEH, Bloem AC. Inability of monoclonal anti-light chain antibody to detect clonal B cells in a patient with follicular lymphoma by multicolor flow cytometry. J Clin Lab Anal;28:493-5.

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