General Product Information

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Monoclonal Antibodies

Dako produces a wide range of monoclonal mouse and monoclonal rabbit antibodies. Each antibody has been carefully selected on the basis of its value, either for research or for the analysis of pathological human cells by immunohistochemistry or flow cytometry.

Tissue Culture Antibodies. With only a few exceptions, Dako monoclonal antibodies are produced in tissue culture. This gives advantages in the use of the antibodies. For example, background problems are virtually absent with such reagents because all the mouse immunoglobulin molecules are directed against the target antigen.

Specificity. Dako monoclonal antibodies are extensively screened on a multitude of tissue sections or other relevant biological material to ascertain that they possess the necessary specificity and give consistent, strong labeling reactions.

Solvent. Dako monoclonal antibodies are, generally, supplied in the liquid form. The majority of unconjugated antibodies are supplied as tissue culture supernatants containing 0.05 mol/L Tris/HCl, pH 7.2, and 15 mmol/L sodium azide. The azide can be removed by dialysis or gel filtration if it interferes with the use of the antibody. However, after removal of the azide, the antibody must be stored frozen.

Storage. 2-8 °C.

Further Information. A package insert is supplied with each vial of monoclonal antibody. It states intended/recommended use, clone, isotype, specificity, as well as recommended staining procedure when applicable. Package inserts are also avaliable on www.dako.com.

The products require no hazard labeling.

Fluorochrome-Conjugated Antibodies for Flow Cytometry

Characterization of Allophycocyanin (APC) Conjugates. Purified monoclonal antibodies or $F(ab')_2$ fragments of affinity-isolated antibodies are conjugated with cross-linked allophycocyanin (APC). After conjugation, unreacted APC and unreacted antibodies are completely removed by gel filtration. The molar APC/antibody ratio is approximately 1. APC conjugates can be excited at 633 nm (HeNe laser) or 635 nm (red diode laser), and emit light at 660 nm.

Characterization of Cascade Yellow (CY) Conjugates. Purified monoclonal antibodies are conjugated with Cascade Yellow (CY)*. After conjugation, unreacted CY is completely removed by gel filtration. The molar CY/ab ratio is approximately 6. CY conjugates can be excited at 406 nm (violet diode laser) and emit light at 541 nm.

Characterization of Fluorescein (FITC) Conjugates. Purified monoclonal antibodies or $F(ab')_2$ fragments of affinity-isolated polyclonal antibodies are conjugated with fluorescein isothiocyanate isomer 1 (FITC). After conjugation, unreacted FITC is completely removed by gel filtration. The molar FITC/ab ratio is approximately 4. FITC conjugates can be excited at 488 nm (blue) and emit light at 530 nm.

Characterization of Pacific Blue (PB) Conjugates. Purified monoclonal antibodies are conjugated with Pacific Blue (PB)*. After conjugation, unreacted PB is completely removed by gel filtration. The molar PB/ab ratio is approximately 6. PB conjugates can be excited at 406 nm (violet diode laser) and emit light at 456 nm.

Characterization of Peridinin Chlorophyll Protein (PerCP) Conjugates. Purified monoclonal antibodies or $F(ab')_2$ fragments of affinity-isolated polyclonal antibodies are conjugated with Peridinin Chlorophyll Protein (PerCP). After conjugation, unreacted PerCP is completely removed by gel filtration. PerCP conjugates can be excited at 488 nm (blue laser) and emit light at 676 nm.

Characterization of Phycoerythrin (RPE) Conjugates. Purified monoclonal antibodies or $F(ab')_2$ fragments of affinity-isolated polyclonal antibodies are conjugated with R-phycoerythrin (RPE). After conjugation, unreacted RPE and unreacted antibodies are completely removed by gel filtration. The molar RPE/antibody ratio is approximately 1. RPE conjugates can be excited at 488 nm (blue) and emit light at 570 nm.

Characterization of Phycoerythrin-Cy5 (RPE-Cy5) Conjugates. Purified monoclonal antibodies are conjugated with an energy transfer fluorochrome (RPE-Cy5) consisting of a cyanine dye, Cy5**, covalently coupled to R-phycoerythrin (RPE). The excitation energy, absorbed at 488 nm by RPE, is transferred to Cy5, which emits light at 670 nm. After conjugation, unreacted RPE-Cy5-complex and unreacted antibodies are completely removed by gel filtration. The molar RPE-Cy5/antibody ratio of the conjugate is approximately 1. Please note that RPE-Cy5 conjugates may bind to monocytes resulting in background staining (1).

MultiMix™ Dual-Color Reagents. Dako MultiMix™ Dual-Color Reagents for flow cytometry are based on the combination of two antibodies labeled with FITC and RPE, respectively. This combination is particularly effective as both fluorochromes can be excited at 488 nm, and the fluorescence emission for FITC is in the green region around 530 nm while the RPE emission is in the orange region above 570 nm. Dako MultiMix™ Dual-Color Reagents are excellent for distinguishing different cell populations simultaneously.

MultiMix™ Triple-Color Reagents. Dako MultiMix™ Triple-Color Reagents for flow cytometry are based on the combination of three antibodies labeled with FITC, RPE and APC, or FITC, RPE and RPE-Cy5, respectively. The Triple-Color Reagents are designed for flow cytometers equipped with a 488 nm (blue) light source for excitation of FITC, RPE and RPE-Cy5, and a 633/635 nm (red) light source for excitation of APC. The antibody and fluorochrome combinations are carefully chosen to provide convenient and reliable reagents for simultaneous identification of specific cell populations.

Performance Testing. All conjugates are thoroughly tested to confirm optimal performance in flow cytometry.

Solvent. The fluorochrome conjugates are offered in liquid form in buffer, containing 15 mmol/L sodium azide and1% bovine serum albumin.

Storage. The conjugates should be stored in the dark at 2-8 °C.

Further Information A package insert is supplied with each vial of conjugate. It provides product-specific details. Package inserts are also available on www.dako.com.

The products require no hazard labeling.

Reference

1. van Vugt MJ, van den Herik-Oudijk IE, van de Winkel JGJ. Binding of PE-Cy5 conjugates to the human high-affinity receptor for IgG (CD64). Blood 1996;88:2358-61.

* The Cascade Yellow™ and Pacific Blue™ antibody conjugates are sold under license from Molecular Probes, Inc., for research use only, except for use in combination with microarrays, and are covered by pending and issued patents.

** Cy5 is trademark of Biological Detection Systems, Inc.