staining for Leu M1 (CD15) in Hodgkin's disease using a secondary antibody specific to IgM. Increased staining intensity with the IgM secondary antibody. There were also a greater number of cases with optimal staining and incubation times due to the higher affinity of the antibody. Therefore, a secondary antibody that is specific to IgM isotype is preferable. An IgG secondary antibody will recognize and cross-react with an IgM heavy chain. However, results are sub-optimal and prolonged incubations are often required. There is a number of monoclonal primary antibodies that are of the IgM isotype (heavy chain). Due to the pentameric structure of IgM, and its incomplete sequence conservation between the heavy chain regions of IgM and IgG isotypes, there are often difficulties obtaining optimal staining results when using an IgM isotype primary antibody with an anti-IgG secondary antibody. An IgG secondary antibody will recognize and cross-react with an IgM heavy chain. However, results are sub-optimal and prolonged incubations are often required.

**Summary & Explanation:**

- The majority of monoclonal primary antibodies for use in immunohistochemistry (IHC) are of the IgG isotype (IgG1, IgG2a, IgG2b, or IgG3). Most IHC detection systems contain a biotinylated secondary (link) antibody that is directed against both the heavy and light chains of mouse IgG. However, there are a number of monoclonal primary antibodies that are of the IgM isotype (heavy chain). Due to the pentameric structure of IgM, and its incomplete sequence conservation between the heavy chain regions of IgM and IgG isotypes, there are often difficulties obtaining optimal staining results when using an IgM isotype primary antibody with an anti-IgG secondary antibody. An IgG secondary antibody will recognize and cross-react with an IgM heavy chain. However, results are sub-optimal and prolonged incubations are often required. Therefore, a secondary antibody that is specific to IgM isotype is preferable. The use of a secondary mouse IgM heavy chain, leads to improved staining characteristics and incubation times can be shortened due to the higher affinity of the reaction. A comparative study by LeBrun et al., demonstrated improved staining intensity with the IgM secondary antibody. There were also a greater number of cases with reactive Hodgkin’s cells that stained with IgM versus IgG secondary antibodies.

**Known Applications:**

- Immunohistochemistry (formalin-fixed paraffin-embedded tissues)

**Supplied As:**

- Affinity-purified Goat anti-Mouse IgM in buffer with Protein carrier and preservative.

**Materials and Reagents Needed But Not Provided:**

- Microscope slides, positively charged
- Desert chamber* (Drying oven)
- Positive and negative tissue controls
- Xylene (Could be replaced with a xylene substitute*)
- Ethanol or reagent alcohol
- Decloaking chamber* (Pressure cooker)
- Deionized or distilled water
- Wash buffer*(TBS/PBS)
- Pretreatment Reagents*
- Enzyme Digestion*
- Avidin-Biotin Blocking Kit* (Labeled Streptavidin Kits Only)
- Peroxidase block*
- Protein block*
- Primary antibody*
- Negative Control Reagents*
- Chromogens*
- Hematoxylin*
- Bluing Reagent*
- Mounting media*

* BIOCARE MEDICAL PRODUCTS: Refer to a BIOCARE MEDICAL Catalog for further information regarding catalog numbers and ordering information. Certain reagents listed above are based on specific application and detection system used.

**Species Reactivity:**

- Mouse IgM heavy chain

**Storage and Stability:**

- Store at 2°C to 8°C. Do not use after expiration date printed on vial. If reagents are stored under conditions other than those specified in the package insert, they must be verified by the user. Diluted reagents should be used promptly; any remaining reagent should be stored at 2°C to 8°C.

**Protocol Recommendations:**

- Deparaffinization:
  - Deparaffinize slides in Slide Brite or xylene. Hydrate slides in a series of graded alcohol to water.

- Peroxide Block:
  - Block for 5 minutes with BIOCARE's PEROXIDAZED 1.

- Pretreatment Solution/Protocol:
  - Please refer to the respective primary antibody datasheet for recommended pretreatment solution and protocol.

- Protein Block:
  - Incubate for 10-15 minutes at RT with BIOCARE's Background Sniper.

- Primary Antibody:
  - Please refer to the respective primary antibody datasheet for incubation time.

- Link:
  - Incubate for 10 minutes at RT with 4plus Goat Anti-Mouse IgM Link.

- Chromogen:
  - Incubate for 10 minutes at RT with 4plus Streptavidin-HRP or Streptavidin-AP.

- Counterstain:
  - Rinse with deionized water. Incubate for 30-60 seconds with Tacha's Automated Hematoxylin. Rinse with deionized water. Apply Tacha's Bluing solution for 1 minute.

**Technical Notes:**

- Use TBS for washing steps.

**Protocol Notes:**

- The optimum antibody dilution and protocols for a specific application can vary due to many factors. These include, but are not limited to: fixation, incubation times, tissue section thickness and detection kit used. The data sheet’s recommendations and protocols are based on exclusive use of BIOCARE products. Due to the superior sensitivity of these unique reagents, the recommended incubation times and titers listed are not applicable to other detection systems, as results may vary. Ultimately, it is the responsibility of the investigator to determine optimal conditions.

**Performance Characteristics:**

- The protocols for a specific application can vary. These include, but are not limited to: fixation, heat-retrieval method, incubation times, tissue section thickness and detection kit used. Due to the superior sensitivity of these unique reagents, the recommended incubation times and titers listed are not applicable to other detection systems, as results may vary. The data sheet recommendations and protocols are based on exclusive use of BIOCARE products. Ultimately, it is the responsibility of the investigator to determine optimal conditions. These products are tools that can be used for interpretation of morphological findings in conjunction with other diagnostic tests and pertinent clinical data by a qualified pathologist.

**Quality Control:**

- Refer NCCLS Quality Assurance for Immunocytochemistry approved guidelines, December 1999 MM4-A Vol.19 No.26 for more information on Tissue Controls.
4plus Biotinylated Goat Anti-Mouse IgM
Affinity-Purified, Biotinylated Goat Anti-Mouse IgM
Detection Component
Control Number: 901-GM603-022608

Precautions:
This product is not classified as hazardous. The preservative used in this reagent is Proclin 950 and the concentration is less than 0.25%. Overexposure to Proclin 950 can cause skin and eye irritation and irritation to mucous membranes and upper respiratory tract. The concentration of Proclin 950 in this product does not meet the OSHA criteria for a hazardous substance. Wear disposable gloves when handling reagents. Specimens, before and after fixation and all materials exposed to them, should be handled as if capable of transmitting infection and disposed of with proper precautions. Never pipette reagents by mouth and avoid contacting the skin and mucous membranes with reagents and specimens. If reagents or specimens come in contact with sensitive areas, wash with copious amounts of water. Microbial contamination of reagents may result in an increase in nonspecific staining. Incubation times or temperatures other than those specified may give erroneous results. The user must validate any such change. The MSDS is available upon request. Consult OSHA, federal, state or local regulations for disposal of any toxic substances.
Proclin™ is a trademark of Rohm and Haas Company, or of its subsidiaries or affiliates.

Troubleshooting:
Follow the reagent specific protocol recommendations according to data sheet provided. If atypical results occur, contact BIOCARE's Technical Support at 1-800-542-2002.

Limitations & Warranty:
There are no warranties, expressed or implied, which extend beyond this description. BIOCARE is not liable for property damage, personal injury, or economic loss caused by this product.