CD138 + Cyclin D1
Prediluted Multiplex Antibody Reagent
901-3193DS-013117

CD138 + Cyclin D1 is a cocktail of mouse and rabbit monoclonal antibodies that is intended for laboratory use in the qualitative identification of CD138 and Cyclin D1 proteins by immunohistochemistry (IHC) in formalin-fixed paraffin-embedded (FFPE) human tissues. The clinical interpretation of any staining or its absence should be complemented by morphological studies using proper controls and should be evaluated within the context of the patient's clinical history and other diagnostic tests by a qualified pathologist.

Summary and Explanation:
Detection of abnormal numbers and/or distribution of bone marrow (BM) plasma cells (PCs) on trephine biopsies can be of importance in the differential diagnosis of multiple myeloma (MM) and other PC neoplasms (1,2). CD138 expression in hematologic malignancy in bone marrow biopsy specimens is limited to nonneoplastic and neoplastic plasma cells and lymphoplasmacytic lymphoma in which strong membrane staining of neoplastic plasma cells is a consistent finding. Other hematopoietic malignancies are CD138 negative (1,2). Some marrow elements, such as stroma, mast cells, histiocytes, fat cells, osteoblasts and osteoclasts, and cells of hematopoietic lineage, are uniformly negative (1,2). Essentially all plasma cells are delineated with a strong membranous immunoreactivity, providing a highly sensitive and specific marker for quantitation of plasma cells in bone marrow biopsies. The reactivity for CD138 is well preserved in both Zenker-fixed and formalin-fixed decalcified tissue biopsies and the use of antigen retrieval do not alter the reactivity of CD138 (1,2). These results demonstrate that CD138 is a highly sensitive and specific marker that is useful for the rapid and precise localization of non-neoplastic and neoplastic PCs on routine BM sections. In addition, because of its clear-cut cell membrane localization, CD138 can be used in double-marker immunostaining reactions to evaluate precisely diagnostic nuclear markers such as Ki67, Cyclin D1, and Cyclin D3 in MMs.

Immunohistochemistry (IHC) and fluorescence in situ hybridization (FISH) are used to investigate Cyclin D1 expression and the presence of chromosome 11 abnormalities in 48 MM patients (40 at diagnosis and 8 at relapse) and in normal plasma cells and hematopoietic precursors in BM biopsies (4). Cyclin D1 overexpression occurred in 12/48 (25%) of cases; combined IHC and FISH analyses in 39 patients showed Cyclin D1 positivity in all of the cases (7/7) bearing the t(11;14), in two of the 13 cases with trisomy 11. Normal plasma cells and other hematopoietic components in BM do not react to Cyclin D1 antibody. The data indicate that the t(11;14) translocation in MM leads to Cyclin D1 overexpression and that IHC analysis may represent a antibody. The data indicate that the t(11;14) translocation in MM leads to Cyclin D1 overexpression and that IHC analysis may represent a

Principle of Procedure Cont'd:
antibody cocktail to the tissue sample, detection is performed by separate secondary antibodies specific for each species (i.e. mouse or rabbit) of the primary antibody cocktail, which are conjugated to horseradish peroxidase (HRP) or alkaline phosphatase (AP) enzymes. Visualization is accomplished by the application of chromogenic substrates (DAB and Warp Red), which are enzymatically activated (by HRP or AP, respectively) to produce a colored reaction product at the antigen site. The specimen may be counterstained and coverslipped. Results are interpreted using a light microscope.

Reagent Provided:
CD138 + Cyclin D1 is provided as a prediluted antibody cocktail of anti-CD138 and anti-Cyclin D1 antibodies in buffer with carrier protein and preservative.

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.

Known Applications:
Immunohistochemistry (formalin-fixed paraffin-embedded tissues)
Species Reactivity: Human; others not tested
Positive Tissue Control: Multiple myeloma, bone marrow or tonsil

Protocol Recommendations:
Peroxide Block:
Block for 5 minutes with Biocare’s Peroxidized 1.

Pretreatment Solution (recommended): Reveal

Pretreatment Protocol:
Heat Retrieval Method:
Preheat the retrieval solution to 95°C for 30 minutes and then place slides in the preheated solution if using Biocare’s Decloaking Chamber Pro or Decloaking Chamber Plus. If using Biocare’s Decloaking Chamber NxGen, place slides into the retrieval solution without preheating. Retrieve at 95°C for 40 minutes. Allow solution to cool for 20 minutes and then wash in distilled water.

Protein Block: Incubate for 10 minutes at RT with Biocare’s Background Punisher.

Primary Antibody: Incubate for 30 minutes at RT.

Double Stain Detection: Incubate for 30 minutes at RT using Biocare’s MACH 2 Double Stain 1.

Chromogen (1): Incubate for 5-7 minutes at RT with Biocare’s Warp Red. Rinse in deionized water.

Chromogen (2): Incubate for 5 minutes at RT with Deep Space Black.

Principle of Procedure:
This product is a primary antibody cocktail of mouse and rabbit antibodies, which may be used in a Multiplex IHC staining procedure to produce a two-color stain. Following application of the primary
Protocol Recommendations Cont’d:

Counterstain:
Counterstain with hematoxylin. Rinse with deionized water. Apply Tacha’s Bluing Solution for 1 minute. Rinse with deionized water.

Technical Note:
This antibody has been standardized with Biocare’s MACH 2 Double Stain 1. Use TBS buffer for washing steps.

Limitations:
The optimum antibody dilution and 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. The clinical interpretation of any positive or negative staining should be evaluated within the context of clinical presentation, morphology and other histopathological criteria by a qualified pathologist. The clinical interpretation of any positive or negative staining should be complemented by morphological studies using proper positive and negative internal and external controls as well as other diagnostic tests.

Quality Control:

Precautions:
1. This antibody contains less than 0.1% sodium azide. Concentrations less than 0.1% are not reportable hazardous materials according to U.S. 29 CFR 1910.1200, OSHA Hazard communication and EC Directive 91/155/EC. Sodium azide (NaN₃) used as a preservative is toxic if ingested. Sodium azide may react with lead and copper plumbing to form highly explosive metal azides. Upon disposal, flush with large volumes of water to prevent azide build-up in plumbing. (Center for Disease Control, 1976, National Institute of Occupational Safety and Health, 1976) (5)
2. 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 into contact with sensitive areas, wash with copious amounts of water. (6)
3. Microbial contamination of reagents may result in an increase in nonspecific staining.
4. Incubation times or temperatures other than those specified may give erroneous results. The user must validate any such change.
5. Do not use reagent after the expiration date printed on the vial.
6. The SDS is available upon request and is located at http://biocare.net.

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

References: