



## Bcl-6 Monoclonal Mouse Anti-Bcl-6 Clone LN22

**REF** 014-1063

**Ready-To-Use** ■ 100 Tests / 50 Tests

**Concentrate** ■ 1mL

### INTENDED USE

**IVD** For in vitro diagnostic use.

Celerus monoclonal mouse anti-Bcl-6, clone LN22, is intended for laboratory use in identifying Bcl-6 positive cells in normal and neoplastic tissue using light microscopy. It may be used with frozen tissue or with formalin-fixed paraffin-embedded tissue.

Positive results aid in the classification of normal and abnormal cells/tissues and serve as an adjunct to conventional histopathology. The clinical interpretation of any positive staining or its absence should be complemented by morphological and histological studies with proper controls. Evaluations should be made by a qualified individual in conjunction with the patient's clinical history and other diagnostic test results.

Refer to the Wave Instrument Operator's Manual for additional information concerning Materials Required but Not Provided; Storage; Staining Procedure; Troubleshooting; Interpretation of Staining; and General Limitations.

### SUMMARY AND EXPLANATION

The Bcl-6 oncoprotein is detected in follicular lymphomas, diffuse large B cell lymphomas, Burkitt's lymphomas, and in nodular, lymphocyte predominant Hodgkin's disease. Anti-Bcl-6 is recommended for use in a panel of antibodies for classifying B cell lymphomas.

### PRINCIPLE OF PROCEDURE

Immunohistochemistry is a multi-step process to identify specific cell markers within tissue biopsies or tumor specimens. The sequential steps include antigen retrieval (optional), antibody application, and antibody visualization followed by optional counterstaining. Specimens are then coverslipped and observed under light microscopy by trained personnel. Normally, multiple antibodies are tested to determine lineage and cell cycle markers. The Celerus Wave is an automated instrument that performs immunohistochemistry stains. For further information on the staining procedure, refer to the Celerus Wave Operator's Manual.

### MATERIALS AND METHODS

#### Reagent Provided

**Clone**  
LN22

**Ig Class**  
IgG<sub>2</sub>, kappa

#### Immunogen

Prokaryotic recombinant protein containing 1-350 amino acids of the N-terminus of the Bcl-6 human oncoprotein molecule.

#### Ready-To-Use in Primary Antibody Cartridge

Celerus anti-Bcl-6 is provided with ProClin 300 as a preservative, in a Primary Antibody Cartridge (PAC), a self-contained dispenser of reagents. Each PAC contains sufficient reagent to complete 100 stained slides. PACs must remain upright to avoid spilling. PAC must be primed before first use. See Celerus Wave Operator's Manual for details.

### Concentrated Antibody

#### Liquid

Liquid concentrated antibody is provided containing 15 mM sodium azide as a preservative and 1% bovine serum albumin as a carrier protein.

#### Lyophilized

Lyophilized antibody is provided containing 15mM sodium azide as a preservative. Reconstitute vial with 1.0 ml distilled water.

#### Dilution

The suggested dilution is 1:10–1:25. This is a guide only and users should determine their own optimal working dilutions.

### SPECIFICITY

Human Bcl-6 oncoprotein.

### MATERIALS REQUIRED BUT NOT PROVIDED

- Wave instrument
- Wave slide rack
- Positively-charged microscope slides, appropriately labeled
- Timer
- Celerus Riptide for antigen retrieval (or equivalent)
- Celerus Target Retrieval Solution (or equivalent)
- Slide drying chamber
- Xylene or xylene substitute
- Reagent alcohol or ethyl alcohol
- Distilled or deionized water
- TBS wash buffer, pH 7.6
- Positive and negative tissue controls
- Celerus Negative Control Reagent (or equivalent)
- Mounting Medium
- Cover slips

### STORAGE AND HANDLING

#### Ready-to-Use PAC, Liquid Concentrated and Lyophilized Antibody

Store reagent at 2-8 °C. Do not freeze. The reagent is stable until the expiration date on the container. Do not use reagent after the expiration date, as the activity cannot be ensured.

#### Reconstituted Antibody

For reconstituted antibody, the reagent is stable for at least two months when stored at 4 °C. For long-term storage it is recommended that aliquots of the antibody be stored at -20 °C. Repeated freezing and thawing of the antibody should be avoided.

There are no signs to indicate instability of this reagent. To ensure a valid staining assay, the use of positive and negative tissue controls is recommended. Contact your Celerus representative if there are stability concerns prior to the expiration date.

### PRECAUTIONS

- For professional users.
- Minimize microbial contamination of reagents or an increase in nonspecific staining may occur.
- As with any product derived from biological sources, proper handling procedures should be used.
- A Material Safety Data Sheet is available for professional users on request

- ProClin 300 is classified per applicable European Community (EC) Directives as: Irritant (Xi). The following are the appropriate Risk (R) and Safety (S) phrases.



- R36** Irritating to eyes  
**R43** May cause sensitization by skin contact  
**S24** Avoid contact with skin  
**S26** In case of contact with eyes, rinse immediately with plenty of water and seek medical advice  
**S35** This material and its container must be disposed of in a safe way  
**S37/39** Wear suitable gloves and eye/face protection  
**S46** If swallowed, seek medical advice immediately and show this container or label.

### WASTE DISPOSAL

Adhere to all local laws when disposing of the PAC.

### PACKAGING DAMAGE

DO NOT USE a PAC if it is leaking, has leaked, has spilled, cannot be primed, or has visually apparent physical damage.

### SPECIMEN COLLECTION AND HANDLING

Formalin-fixed paraffin embedded (FFPE) tissues, frozen tissues, or smears are suitable for use. Wave detection kits have been optimized for tissues fixed with 10% formalin. Ideally, each 4-6µ tissue section should be placed on charged slides on the lower 2/3 of the slide. Very large sections should be placed 1/4 inch below the lower end of the slide label.

Slides should be baked overnight at 37 °C, or at 60 °C for one hour.

Use standard histochemical techniques to deparaffinize processed slides. For uniformity of staining results, it is recommended that target retrieval be performed using the Celerus Riptide and Celerus Target Retrieval Solution (or equivalent) at 112 °C for 5 minutes. Insert slides into the Wave Instrument according to the staining grid provided by the instrument software. Avoid drying of the tissue specimen during this process. After all slides to be stained have been inserted and reagents mounted on the instrument, start the staining run.

When the slides have completed the staining run, remove them from the instrument, coverslip, and view under light microscopy.

### PRODUCT-SPECIFIC LIMITATIONS

Bcl-6 antibody, when used on the Wave instrument, detects antigens that survive routine formalin fixation, tissue processing, and sectioning. Users who deviate from recommended test procedures are responsible for interpretation and validation of patient results.

### RESULTS EXPECTED/ PERFORMANCE CHARACTERISTICS

#### Normal Tissues

The antibody detected Bcl-6 protein in the nuclei of germinal center B cells. Weak and variable staining of epithelia and spindle-shaped stromal cells was observed in some tissues.

#### Abnormal Tissues

The antibody detected 18/19 diffuse large B cell lymphomas, 11/11 follicular lymphomas, 6/9 Hodgkin's lymphomas, 2/2 Burkitt's lymphomas, 1/4 anaplastic large cell lymphomas, and 1/3 mantle cell lymphomas. Staining was also observed in 4/4 Castleman's disease. No staining was seen in 7/7 T-cell lymphomas, 5/5 T lymphoblastic lymphomas, 3/3 NK/T cell lymphomas, 3/3 MALT lymphomas, 2/2 marginal zone B cell lymphomas, a monocytoid B cell lymphoma, and a variety of non-lymphoid tumors (n=64).

### REFERENCES

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Lo Coco F, Ye BH, Lista F, et al. Rearrangements of the BCL-6 gene in diffuse large-cell non-Hodgkin's lymphoma. *Blood* 83(7):1757-1759, (1994).

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