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Bcl-2 Ready-To-Use IHC Kit

Cat.No: IHC0280
Applications: **IHC-P**
Reactivity: Human, Mouse, Rat
Size: 50T
Assay type: Immunohistochemistry
Sample type: FFPE tissue
General Information:

Number	Component	Size	Concentration	Storage
1	PBS Buffer (powder)	2 L×2	20x	RT
2	Antigen Retrieval Buffer	20 ml	100x	2-8°C
3	Endogenous Peroxidase Blocking Buffer	3 ml	RTU	2-8°C, protect from light
4	Blocking Buffer	3 ml	RTU	2-8°C
5	Primary Antibody (Bcl-2 Recombinant Rabbit mAb)	6 ml	RTU	2-8°C
6	Secondary Antibody (Goat Anti-Rabbit IgG H&L, HRP conjugated)	6 ml	RTU	2-8°C
7	Chromogen Component A	0.3 ml	RTU	-20°C,protect from light
8	Chromogen Component B	0.3 ml	RTU	-20°C
9	Counter Staining Reagent	5 ml	RTU	RT
10	Mounting Media	5 ml	RTU	RT
11	Control slide (Human tonsil, rat spleen, mouse spleen)	3 slides	RTU	RT
12	Datasheet	1 copy		

Storage and Stability: Please store components at the temperatures indicated on the individual tube labels. The kit is stable for 6 months from the date of receipt.

Immunohistochemistry Protocol:

1. **Deparaffinization And Rehydration**

Immerse slides in fresh xylene for 15 minutes and then repeat two more times using separate containers. Immerse slides sequentially in 100%, 95%, 90%, 80%, and 70% ethanol solutions for 5 minutes each. Rinse slides 3 times with distilled water for 5 minutes each.

2. **Antigen Retrieval**

Add 100× **Antigen Retrieval Buffer** into distilled water to prepare a 1×solution. Boil slides in 1×solution at 95°C-100°C for 15 minutes. Move the slides to 1×solution at room temperature (RT) and allow them to stand for 20 minutes. Rinse 3 times with **PBS Buffer** (dissolve the powder in 2L distilled water) for 5 minutes each.

3. **Block Endogenous Peroxidase**

Drain the liquid off the slides and then use a hydrophobic IHC pen to draw circles on the slides around tissue sections. Add 2-4 drops of **Endogenous Peroxidase Blocking Buffer** directly on slides, covering the whole tissue and block slides for 15 minutes at RT. Rinse 3 times with **PBS Buffer** for 5 minutes each.

4. **Serum Blocking**

Block with 2-4 drops of **Blocking Buffer** for 20 minutes at RT.

5. **Primary Antibody Incubation**

Drain blocking buffer from slides. Incubate slides with 2-4 drops of **Bcl-2 Recombinant Rabbit mAb** overnight at 4°C or 1-2 hours at RT. Rinse 3 times with **PBS Buffer** for 5 minutes each.

6. **Secondary Antibody Incubation**

Incubate slides with 2-4 drops of **Goat Anti-Rabbit IgG H&L, HRP conjugated** for 1-2 hours at RT. Rinse slides 3 times with **PBS Buffer** for 5 minutes each.

7. **Signal Development**

Remove residual liquid around the tissue section. Add 50ul fresh **DAB Buffer** (**Chromogen Component A : Chromogen Component B : PBS Buffer=1:1:18**) to cover the tissue. Monitor the reaction under the microscope until a brown color is visible (approximate 3-5 minutes at RT). Stop reaction immediately by rinsing with distilled water. Rinse slides 3 times with distilled water for 5 minutes each.

8. **Counterstain**

Counterstain with an appropriate amount of **Counter Staining Reagent** for 3-5 minutes at RT. Rinse slides with distilled water for 5 minutes. Use 2-4 drops of **Differentiation reagent** to cover the tissue for 30 seconds. Rinse slides twice with distilled water for 5 minutes each.

9. **Dehydration Sheet**

Immerse slides sequentially in 70%, 80%, 90%, 95%, and 100% ethanol for 5 minutes each at RT. Immerse slides in 2 changes of fresh xylene, 15 minutes each. Drop some **Mounting Media** on the tissue. Mount coverslips.

Notes:

1. The positive control slide provided in the kit allows you to be sure that the experimental set-up is working properly.
2. Do not allow slides to dry at any time during this procedure.

3. Please don't replace the matching reagents in this product with other manufacturers' products.

4. As DAB is a carcinogen, please take necessary precautions.

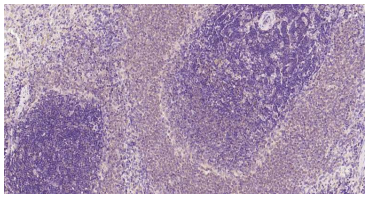
5. PBS (reagent 1) can be stored for one week at 4°C after preparation; The antigen retrieval buffer (1×reagent 2) and the chromogenic agent (the mixture of reagents 7 and 8) should be prepared right before each assay.

Please cite this product as " IHC0280, Bioss Antibodies". Citation example: " Tissue sections using BCL2 IHC Kit (IHC0280, Bioss Antibodies) were stained for BCL2 according to the manufacturer's instructions."

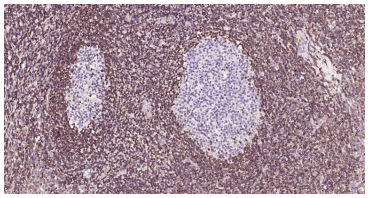
Introduction:

BCL-2 is a key regulator of apoptosis that functions to either inhibit or promote cell death. The BCL-2 family members are also characterized by dimerizing to further modulate apoptosis. Bag1, for example, has been found to form a heterodimer with BCL-2 resulting in the enhancement of the anti-apoptotic effect of BCL-2. Bax and Bak have been shown to play a critical role in cytochrome c release from mitochondria and thus initiate apoptosis. Bax exerts a pro-apoptotic rather than an anti-apoptotic effect on cells. Constitutive expression of BCL2, such as in the case of translocation of BCL2 to Ig heavy chain locus, is thought to be the cause of follicular lymphoma. In most follicular lymphomas, neoplastic germinal centers express high levels of BCL-2 alpha protein, whereas the normal or hyperplastic germinal centers are negative. Two transcript variants of BCL-2, produced by alternate splicing, differ in their C-terminal ends. The overexpression of BCL-2 has been linked to human cancers such as B-cell lymphoma and prostate cancer.

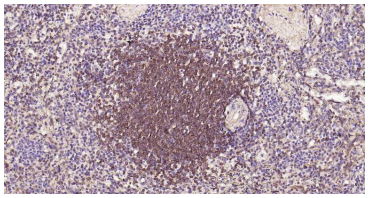
Validation Data



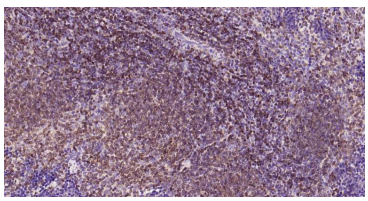
Immunohistochemical analysis of paraffin embedded rat spleen tissue slide using IHC0280 (Bcl-2 IHC Kit).



Immunohistochemical analysis of paraffin embedded human tonsil tissue slide using IHC0280 (Bcl-2 IHC Kit).



Immunohistochemical analysis of paraffin embedded human spleen tissue slide using IHC0280 (Bcl-2 IHC Kit).



Immunohistochemical analysis of paraffin embedded mouse spleen tissue slide using IHC0280 (Bcl-2 IHC Kit).

PRODUCT SPECIFIC PUBLICATIONS

[IF=1.2] Xiaoxuan Hu. et al.Mechanisms of Apoptosis and Pulmonary Fibrosis Resulting From Sulfur Mustard-Induced Acute Pulmonary Injury in Rats.INTERNATIONAL JOURNAL OF TOXICOLOGY.2025 Jan 31:10915818251315907. IHC ; Rat . 39888856