

**Certificate of Analysis**

**APOPTOPAK Miniature Set**  
**Catalog # 17-178**  
**Lot # 22510**

Antibody	Anti-Bcl-2, clone 124 (mouse monoclonal IgG <sub>1</sub> )	Anti-Bak, NT (rabbit polyclonal IgG)	Anti-Bax, NT (rabbit polyclonal IgG)
Catalog #	05-341-MN	06-536-MN	06-499-MN
Lot #	21644A	22508	21872
[Immunoblot]	use 0.5-2µg/ml	use 0.5-2µg/ml	use 0.5-2µg/ml
Amount	25µg in 25µl	25µg in 25µl	40µg in 40µl
Physical Form	Frozen liquid	Frozen liquid	Liquid
Storage & Stability	Stable for 2 years at -20°C from the date of shipment	Stable for 2 years at -20°C from the date of shipment	Stable for 2 years at -20°C from the date of shipment
Immunogen	amino acids 41-54 of human Bcl-2	amino acids 23-37 of human Bak (SEEQVAQ QDTEEVFRS-C) with a cysteine added to the C- terminus	Amino acids 1-21 of human Bax (MSDGSGE QPRGGGPTSSEQIMK-C) with a cysteine added to the C-terminus
Purification	protein G	protein A	protein A
Known Species Cross- Reactivity	mouse, human	mouse, human	mouse, human
Additional Applications	IP, ICC, IHC	IP	IP, ICC

**Antibodies also available individually:**

Antibody	Catalog #	Pack Size
Anti-Bcl-2	05-341	100µg
Anti-Bak, NT	06-536	200µg
Anti-Bax, NT	06-499	200µg

**FOR IN VITRO RESEARCH USE ONLY**  
**NOT FOR USE IN HUMANS OR ANIMALS**

### **Immunoblot Protocol**

1. Perform SDS-polyacrylamide gel electrophoresis (SDS-PAGE) on a cell lysate sample (cell lysis buffer: 50mM Tris-HCl, pH7.4; 1% NP-40; 0.25% sodium deoxycholate; 150mM NaCl; 1mM EGTA; 1mM PMSF; 1µg/ml aprotinin, leupeptin, pepstatin; 1mM Na<sub>3</sub>VO<sub>4</sub>; 1mM NaF) and transfer the proteins to nitrocellulose. Wash the blotted nitrocellulose twice with water.
2. Block the blotted nitrocellulose in freshly prepared 3% nonfat dry milk (Catalog # 20-200) in PBS (PBS-MLK) for 20 minutes at 20-25°C with constant agitation.
3. Incubate the nitrocellulose with the indicated amount of primary antibody, diluted in freshly prepared PBS-MLK overnight with agitation at 4°C.
4. Wash the nitrocellulose twice with water for 5 minutes.
5. Incubate the nitrocellulose in the secondary reagent of choice in PBS-MLK for 1.5 hours at room temperature with agitation.
6. Wash the nitrocellulose with water for 5 minutes twice.
7. Wash the nitrocellulose in PBS-0.05% Tween 20 for 3-5 minutes.
8. Rinse the nitrocellulose in 4-5 changes of water.
9. Use detection method of choice (enhanced chemiluminescence was used).