

## Product Information

### Bcl-w (Minus C-Terminus)

Human, Recombinant  
Expressed in *Escherichia coli*

Product Number **B 1059**

#### Product Description

Recombinant Human Bcl-w (Minus C-Terminus) is produced from a cDNA sequence corresponding to amino acid 1-172 of human Bcl-w (missing the 17 amino acid C-terminal and containing a histidine tag on the C-terminal). The accession number for the sequence is U59747. It is expressed in *Escherichia coli* then purified from the soluble fraction of disrupted cells. Detergents were not used in the purification process.

Recombinant Human Bcl-w (Minus C-Terminus) migrates as a polypeptide of 18 kDa on SDS-PAGE under both reducing and non-reducing conditions. A band corresponding to a disulfide-linked dimer is observed with non-reducing PAGE. Amino acid sequencing indicates that the N-terminal methionine is removed during expression in *Escherichia coli*.

Bcl-w is a member of the Bcl-2 family of proteins that regulates outer mitochondrial membrane permeability.<sup>1,2</sup> At least fifteen Bcl-2 family members have been identified in mammalian cells. Pro-survival members of the Bcl-2 family, which include Bcl-X<sub>L</sub>, Bcl-X, Mcl-1 and A1, inhibit apoptosis in response to a wide variety of cytotoxic insults, whereas the pro-apoptotic family members (e.g. Bax, Bak, Bad, Bik and Bid) in general antagonize the function of the pro-survival family members.

Bcl-w is an anti-apoptotic member that prevents release of cytochrome c from the mitochondria intermembrane space into the cytosol. It is required for normal sperm maturation.<sup>3,4</sup> Natural Bcl-w contains a carboxyl-terminal mitochondria targeting sequence. Recombinant Bcl-w lacks the mitochondrial targeting sequence but maintains its ability to neutralize pro-apoptotic Bcl-2 family members. Neutralization by Bcl-w appears to occur through binding the BH3 region of pro-apoptotic Bcl-2 family members. This activity does not require the mitochondrial targeting sequence.

#### Reagent

Recombinant Human Bcl-w (Minus C-Terminus) is supplied as approximately 50 µg of protein in a 0.2 µm filtered solution in 25 mM HEPES, pH 7.5, and 0.1 M KCl, and 10% glycerol.

Concentration is approximately 1.0 mg/ml

#### Storage/Stability

Store at -20 °C. Sterile Bcl-X<sub>L</sub> can be stored at 2-8 °C for up to one week. For prolonged storage, freeze in working aliquots at -20 °C. Avoid repeated freezing and thawing. Do not store in a frost-free freezer.

No change in EC<sub>50</sub> or dimerization has been observed in recombinant human Bcl-w that is stored at -20 °C.

#### Product Profile

Recombinant human Bcl-w is assayed for its ability to inhibit the release of cytochrome c from isolated mouse liver mitochondria induced by caspase-8 cleaved human Bid (Sigma product No. C 4608) using a mouse/rat cytochrome c ELISA assay.

The EC<sub>50</sub> for human Bcl-w in this assay ranges from 50 to 250 nM in the presence of 54 nM of caspase 8-cleaved human BID.

Each laboratory should determine the dose response of the desired application.

Purity: > 95% by SDS-PAGE visualized by silver staining.

Greater than 75% of human Bcl-w elutes at a position corresponding to 18 kDa on size exclusion chromatography, indicating that greater than 75% of recombinant human Bcl-w is monomeric. The remainder exists as a disulfide-linked dimer.

**References**

1. Gross, A. et al., Genes and Develop., **13**, 1899 (1999).
2. Kroemer, G., Nature Med., **3**, 614 (1997).
3. Ross, J.A., et al., Nat. Genet., **18**, 251 (1998).
4. Yan, W., et al., Mol. Endocrin., **14**, 682 (2000).

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