Invitrogen® Bcl-2 ELISA Kit

Cat. No. 99-0042
Size: 96 Tests
For Research Use Only
Lot No.

INTENDED USE

Invitrogen® Bcl-2 ELISA Kit is an enzyme-linked immunosorbent sandwich assay for the quantitative detection of human Bcl-2 in cell lysates, supernatants, whole blood, and serum.

INTRODUCTION

Bcl-2, encoded by a proto-oncogene, is an intracellular membrane-associated protein that functions to block programmed cell death.[6] Expression of Bcl-2 has been reported in a wide range of hematopoietic cells, nonneoplastic epithelia and epithelial malignancies. The Bcl-2 gene has a unique function among mammalian oncogenes as a negative regulator of apoptosis.[9] Bcl-2 is associated with stem cells and committed to differentiation and morphogenesis. Several homologs of Bcl-2 have recently been discovered, and some of which function as inhibitors of cell death and others as promoters of apoptosis that oppose the function of the Bcl-2 protein.[4]

Bcl-2 protein plays a critical role in oncogenesis and resistance to cancer therapy. Furthermore prognostic significance of Bcl-2 expression has been shown for several malignancies such as Non-Hodgkin's Lymphoma[3], squamous cell carcinomas[10], breast carcinoma[1], gallbladder carcinoma[12], and thymoma[2]. Deregulated Bcl-2 expression has been shown in Multiple Myeloma patients[7] and in acute myeloid leukemia (AML)[5]. Bcl-2 has been suggested as a useful marker for adequate IL-2 therapy in AIDS patients.[8]

REAGENTS AND MATERIALS PROVIDED

1 Coated Microwell Plate (coated with Monoclonal Antibody (murine) to human Bcl-2)
1 vial Biotin-Conjugate anti-Bcl-2 (100 µL)
1 vial Streptavidin-HRP (150 µL)*
2 vials Bcl-2 Standard Lyophilized
1 bottle 10x Lysis Buffer (15 mL)
1 bottle 20x Wash Buffer (50 mL) (PBS with 1% Tween 20)*
1 vial 20x Assay Buffer (5 mL) (PBS with 1% Tween 20 and 10% BSA)*
1 bottle Sample Diluent (12 mL)*
1 vial Substrate Solution (15 mL)
1 vial Stop Solution (12 mL) (1M Phosphoric acid)
1 vial each Green-Dye, Red-Dye, Blue-Dye (0.4 mL each)
4 adhesive Plate Covers
* Reagents containing thimerosal as preservative may be toxic if ingested

MATERIALS REQUIRED BUT NOT PROVIDED

5 mL and 10 mL graduated pipettes, beakers, flasks, and cylinders
10 µL to 1,000 µL adjustable single channel micropipettes with disposable tips
50 µL to 300 µL adjustable multichannel micropipette, disposable tips, and reservoir
Microwell strip reader capable of reading at 450 nm (620 nm as optional reference wave length)

STORAGE

2° - 8°C

PRINCIPLE OF TEST

Protein of interest in the samples and standards binds to the antibody coated on the plate. A biotin-conjugated antibody is added and binds to protein captured by the first antibody. Streptavidin-HRP is added and binds to the biotin-conjugated antibody. The substrate solution is added to the wells to form the colored products. The reaction is then terminated by addition of acid and absorbance is measured at 450 nm. A standard curve is prepared to determine the protein concentration.

PREPARATION OF REAGENTS

A. Wash Buffer: If crystals have formed in the Wash Buffer Concentrate, warm it gently until they have completely dissolved. Add 50 mL of the 20x Wash Buffer into 950 mL distilled or deionized water. Mix gently to avoid foaming. The pH of the final solution should be adjusted to 7.4. Store at 2° to 25°C. The Wash Buffer is stable for 30 days.

B. Assay Buffer: Mix the Assay Buffer well before use. Add 5 mL of 20x Assay Buffer to 95 mL distilled or deionized water. Mix gently to avoid foaming. Store at 2° to 8°C. The Assay Buffer is stable for 30 days.

C. Preparation of Biotin-Conjugate: Make 1:100 dilution with Assay Buffer as needed (50 µL/reaction).

D. Preparation of Standard: Reconstitute standard by addition of distilled water. Reconstitution volume is stated on the label of the standard vial. Swirl gently. Store reconstituted Standard promptly at –20°C after use. Discard after one week.

E. Preparation of Streptavidin-HRP: Make 1:100 dilution with Assay Buffer as needed (100 µL/reaction).

F. Addition of color-giving reagents: Green-Dye, Red-Dye, Blue Dye.

1. Biotin-Conjugate: Before dilution of the concentrated Biotin-conjugate, add the Green-Dye at a dilution of 1:100.
2. Streptavidin-HRP: Before dilution of the concentrated Streptavidin-HRP, add the Red-Dye at a dilution of 1:250.
3. Sample Diluent: Before dilution of the sample, add Blue-Dye at a dilution of 1:250.

SAMPLE PREPARATION

Cell and tissue culture lysis procedure (cell culture samples)

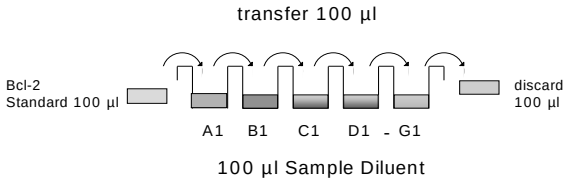
a. For suspension cells, pellet the cells by spinning down the cells at 1200 x g for 15 min. Remove supernatant.
b. For attached cells, remove supernatant from cell culture, wash cells once with PBS, harvest cells by scraping and gentle centrifugation.
c. Aspirate PBS leaving an intact cell pellet (at this time the cell pellet can be frozen at –80°C and lysed at a later date). Resuspend cells in Lysis Buffer to a concentration of 5 x 10^6 cells/mL.
d. Incubate 60 min. at room temrerature with gentle shaking.
e. Transfer extracts to microfuge tubes and centrifuge at 1000 x g for 15 min.
f. Aliquot cleared lysate to clean microfuge tubes. Lysates are now ready for analysis, or can be stored at –80°C.

Note: Samples found to contain greater than 32 ng/mL Bcl-2 must be diluted with Sample Diluent (provided) so that the Bcl-2 concentration falls within the range spanned by the standard curve, and assayed again.

PROCEDURE

1. Mix all reagents thoroughly without foaming before use.
2. Wash the microwells twice with approximately 300 µL Wash Buffer per well with thorough aspiration of microwell contents between washes. Take care not to scratch the surface of the microwells. After the last wash, empty the wells and tap microwell strips on absorbent pad or paper towel to remove excess Wash Buffer. Use the microwell strips immediately after washing or place upside down on a wet absorbent paper for not longer than 15 minutes. Do not allow wells to dry.
3. Add 100 µL of Sample Diluent to all standard wells and to the blank wells. Add 100 µL Bcl-2 standard to first standard wells. Prepare serial 1:2 dilutions of standard, in duplicate, ranging from 32 ng/mL to 0.5 ng/mL as shown in Figure 1.

Figure 1. Preparation of Bcl-2 standard dilutions:



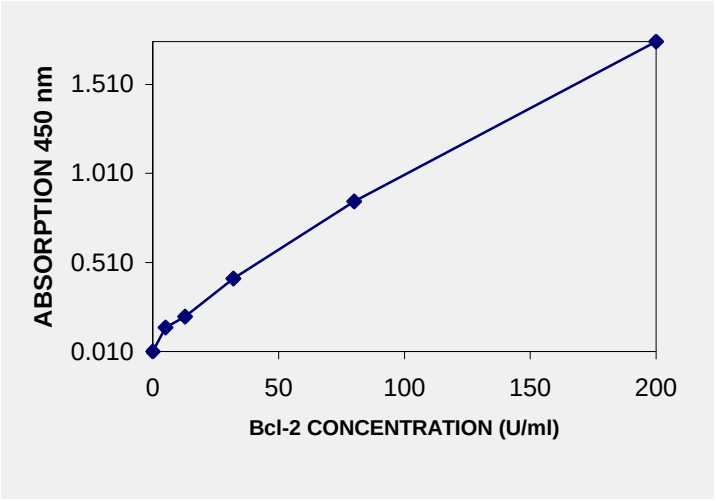
4. Add 100 µL of Sample Diluent, in duplicate, to the blank wells.
5. Add 80 µL of Sample Diluent, in duplicate, to the sample wells.
6. Add 20 µL of each Sample, in duplicate, to the designated wells.
7. Add 50 µL of diluted biotin-conjugate to all wells, including the blank wells.
8. Cover with a plate cover and incubate at room temperature, on a microplate shaker at 100 rpm if available, for 2 hours.
9. Remove plate cover and empty the wells. Wash microwell strips 3 times as described in step 2.
10. Add 100 µL of diluted Streptavidin-HRP to all wells, including the blank wells.
11. Cover with a plate cover and incubate at room temperature, on a microplate shaker at 100 rpm if available, for 1 hour.

12. Remove plate cover and empty the wells. Wash microwell strips 3 times as described in step 2. Proceed to the next step.
13. Pipette 100 µL of mixed TMB Substrate Solution to all wells, including the blanks.
14. Incubate the microwell strips at room temperature (18° to 25°C) for about 15 minutes, if available on a rotator set at 100 rpm. Avoid direct exposure to intense light. The point, at which the substrate reaction is stopped, is often determined by the ELISA reader. Many ELISA readers record absorbance only up to 2.0 O.D. Therefore the color development within individual microwells must be watched by the person running the assay and the substrate reaction stopped before positive wells are no longer properly detectable.
15. Stop the enzyme reaction by quickly pipetting 100 µL of Stop Solution into each well, including the blank wells. It is important that the Stop Solution is spread quickly and uniformly throughout the microwells to completely inactivate the enzyme. Results must be read immediately after the Stop Solution is added or within one hour if the microwell strips are stored at 2 - 8°C in the dark.
16. Read absorbance of each microwell on a spectrophotometer using 450 nm as the primary wave length (optionally 620 nm as the reference wave length; 610 nm to 650 nm is acceptable).

CALCULATION OF RESULTS

1. Calculate the average absorbance for each set of duplicate standards and samples. Duplicates should be within 20 per cent of the mean.
2. Create a standard curve by plotting the mean absorbance for each standard on the Y-axis and the protein concentration on the X-axis. Draw a best-fit curve through the points.
3. The protein concentration for each sample can be determined using the standard curve.
4. For the diluted samples, the concentration read from the standard curve must be multiplied by the dilution factor.
5. It is suggested that a control sample of known concentration is run in each assay.
6. A representative standard curve is shown in Figure 2. This curve cannot be used to determine test results.

Figure 2. Representative standard curve for Bcl-2 ELISA



PERFORMANCE CHARACTERISTICS

A. Sensitivity

The limit of detection of Bcl-2 defined as the analyte concentration resulting in an absorbance significantly higher than that of the dilution medium (mean plus three standard deviations) was determined to be < 0.5 ng/mL (mean of 6 independent assays).

B. Reproducibility

I. Intra-assay

Assay was carried out with 6 replicates of 8 samples containing different concentrations of Bcl-2. The overall intra-assay coefficient of variation was calculated to be 8.6%.

Table 1. Intra-assay

Sample	Experiment	Bcl-2 Con (ng/mL)	CV (%)
1	1	87.9	5
	2	76.6	9
	3	64.3	4
2	1	63.4	7
	2	54.4	9
	3	47.9	9
3	1	54.0	14
	2	40.8	9
	3	40.0	14
4	1	44.0	11
	2	35.3	7
	3	34.8	12
5	1	42.0	4
	2	46.1	4
	3	53.8	9
6	1	16.0	10
	2	19.8	7
	3	16.7	10
7	1	7.4	6
	2	7.7	11
	3	8.3	10
8	1	5.0	11
	2	5.5	7
	3	5.3	7

II. Inter-assay

Each assay was carried out with 6 replicates of 8 samples containing different concentrations ofBcl-2. The overall inter-assay coefficient of variation was calculated to be 12.0 %.

Table 2. Inter-assay

Sample	Bcl-2 Conc. (ng/mL)	CV (%)
1	75.3	15.8
2	55.2	14.0
3	44.8	17.7
4	38.0	13.5
5	47.3	12.6
6	17.5	11.6
7	7.8	5.9
8	5.3	5.1

C. Spike Recovery

The spike recovery was evaluated by spiking four levels of Bcl-2 into Lysis buffer. Mean recovery was 71%.

D. Dilution Parallelism

Bcl-2 positive samples with different levels of Bcl-2 were analyzed at serial dilutions for 1:5 to 1:40. The overall recovery was 102 %.

E. Sample Stability

I. Freeze-Thaw Stability

Aliquots of samples were stored frozen at –20°C and thawed up to 5 times, and Bcl-2 levels determined. There was no significant loss of Bcl-2 by freezing and thawing up to 5 cycles of freezing and thawing.

II. Storage Stability

Aliquots of samples were stored at –20°C, 2-8°C, room temperature (RT) and 37°C and the Bcl-2 levels were determined after 24 hours. There was no significant loss of Bcl-2 immunoreactivity when stored at –20°C.

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RELATED PRODUCTS

Product Name	Clone/PAD*	Cat. No.
Mouse anti-Bcl-2	Bcl-2-100	13-8800
Mouse anti-Bcl-2	Bcl-2-100	18-0193
Mouse anti-Bcl-2	10C4	33-6100

*PAD: Polyclonal Antibody Designation