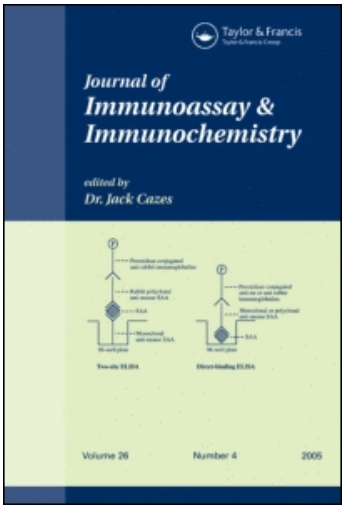


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# Development, Evaluation, and Application of a Highly Sensitive Microtiter Plate ELISA for Human Bcl10 Protein

Sumit Bhattacharyya[a]; Nitika Pant[a]; Pradeep K. Dudeja[a]; Joanne K. Tobacman[a]
[a] Department of Medicine, University of Illinois at Chicago and Jesse Brown VA Medical Center, Chicago, IL, USA

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# Development, Evaluation, and Application of a Highly Sensitive Microtiter Plate ELISA for Human Bcl10 Protein

**Sumit Bhattacharyya, Nitika Pant, Pradeep K. Dudeja, and Joanne K. Tobacman**
Department of Medicine, University of Illinois at Chicago and Jesse Brown VA Medical Center, Chicago, IL, USA

**Abstract:** Bcl10 (B-cell CLL/lymphoma 10) is a 233 amino acid CARD (caspase recruitment domain)-containing cellular protein, increasingly recognized as a mediator of NF$\kappa$B activation in non-immune, as well as immune cells. Due to the importance of Bcl10 in diverse cell types, we developed a solid-phase, enzyme-linked immunosorbent (ELISA) assay to precisely measure Bcl10 in small volume cell lysates, using recombinant Bcl10 to standardize the assay. Standard curve measures Bcl10 from 0.25 ng/mL to 16 ng/mL, with very low intra- and inter-assay variation. Sample dilution and exogenous Bcl10 recovery experiments, comparisons with Western blot, and linear response to increasing doses of known Bcl10 activators confirm the specificity and precision of the ELISA.

**Keywords:** Bcl10, ELISA, NF$\kappa$B, Inflammation, Apoptosis, CARD

## INTRODUCTION

B-cell lymphomas of mucosa-associated lymphoid tissue (MALT lymphomas) arise in extranodal sites, most commonly the stomach.[1] They are characterized by a recurrent breakpoint upstream of the Bcl10 promoter. Cytogenetic studies of low-grade malignant MALT lymphomas have identified translocations, including t(1;14)(p22;q32) and t(11;18)(q21;q21), that are associated with constitutive activation of NF$\kappa$B.[2,3] Bcl10 has been identified as the cellular homolog of the equine herpesvirus-2 E10 gene, and

Address correspondence to Joanne K. Tobacman, MD, Department of Medicine, University of Illinois at Chicago, Chicago, IL 60612, USA. E-mail: jkt@uic.edu

associated with apoptosis and propensity for malignant transformation when mutated, as well as NF$\kappa$B activation.[3–9]

The Bcl10 gene encodes for a cytosolic protein of 233 amino acids and contains an amino-terminal caspase recruitment domain (CARD), homologous to that found in several pro-apoptotic molecules.[3] Willis et al. found that Bcl10 expressed in a MALT lymphoma exhibited a frame shift mutation, resulting in truncation distal to the CARD domain, which was associated with activation of NF$\kappa$B, but not apoptosis.[3] Bcl10 is widely expressed in the cytoplasm of normal lymphoid tissues, with the expression level depending on the developmental stage of lymphocytes.[10] In contrast to normal B cells that express Bcl10 in the cytoplasm, MALT lymphoma cells express Bcl10 protein predominantly in the nucleus.[11–13] When Xue et al. generated healthy, fertile transgenic mice lacking Bcl10, the mice were unable to activate NF$\kappa$B in response to lipopolysaccharide.[14] Mutant mice did not survive infection with *Streptococcus pneumoniae*, suggesting a vital role for Bcl10 in development of mature B-cell subsets.[14]

Recent reports have determined the role of Bcl10 in an inflammatory cascade in intestinal epithelial cells, embryonic fibroblasts, embryonic kidney cells, and hepatocytes.[15–18] Hence, Bcl10 emerges as a significant proapoptotic and proinflammatory protein, in both immune and non-immune cells. We have identified Bcl10 as a critical mediator of carrageenan-induced activation of NF$\kappa$B and IL-8 in normal human intestinal epithelial cells.[15]

With increasing recognition of the role of Bcl10 in mediation of NF$\kappa$B activation in non-immune cells, as well as in lymphocytes and macrophages, accurate, quantitative measurements of Bcl10 protein have become increasingly important. In order to quantify Bcl10 under various experimental conditions, we have developed a sensitive and specific solid-phase ELISA (enzyme-linked immunosorbent assay) for precise measurement of Bcl10. The features of this new solid-phase ELISA are presented in this report.

## EXPERIMENTAL

### Materials

Polyclonal rabbit antibody to Bcl10 (QED Biosciences Inc., San Diego, CA), recombinant human Bcl10 (Calbiochem, EMD Biosciences, San Diego, CA), mouse monoclonal antibodies to Bcl10 (Novus Biologicals, Littleton, CO and Santa Cruz Biotechnology, Santa Cruz, CA) and anti-mouse IgG-HRP conjugate (Santa Cruz) were obtained from commercial sources. High binding microtiter plates and tetramethylbenzidine (TMB)/$H_2O_2$ substrate were obtained (R&D Systems, Minneapolis, MN). All other chemicals were obtained from Sigma Chemical Co. (St Louis, MO).

NCM460 cells (INCELL, San Antonio, TX) were grown in specialized M3:TM media as previously described.[15,19] Normal human intestinal

epithelial cells were obtained from colonic surgeries in accord with a protocol approved by the University of Illinois at Chicago Institutional Review Board as previously reported.[15] Other cell lines, including Caco2, IB3, C38, and MCF-7 cells were obtained from American Type Culture Collection (ATCC, Manassas, VA) and grown under the recommended conditions.

## Methods

### Microtiter Plate Coating

A polyclonal rabbit antibody to Bcl10 was procured (QED Biosciences Inc., USA) and diluted with PBS (137 mM NaCl, 2.7 mM KCl, 8.1 mM $Na_2HPO_4$ and 1.5 mM $KH_2PO_4$ at pH 7.4) to concentrations of 1, 2 and 4 μg/mL for coating the microtiter plate. This antibody recognizes and binds to amino acids 5–19 of human Bcl10 peptide. 100 μL of antibody was added to each well of the microtiter plate (R&D Systems), sealed, and incubated at room temperature (RT) overnight in order to coat the microplate. 4 μg/mL concentration of this antibody was found to work best to capture cellular Bcl10, and was subsequently used for all assays.

### Non-Specific Blocking

Following overnight incubation, the excess antibody solution in the plates was aspirated, and the wells were washed three times with 250 μL of Wash Buffer (0.05% Tween 20 in PBS at pH 7.4). After washing, non-specific blocking was performed by adding 250 μL of Blocking Buffer (2% BSA in PBS) to each well and incubating for 1 hour at RT. After blocking, the plate was washed three times with Wash Buffer.

### Preparation of the Samples

Cells were washed with ice cold PBS twice, and then the cells were pelleted by centrifugation at 2000 g for 5 minutes and PBS removed. Pellets were resuspended in cell lysate buffer (20 mM Tris-HCl at pH 7.5, 150 mM NaCl, 1 mM EDTA, 1 mM EGTA, 1% Triton X-100, 2.5 mM sodium pyrophosphate, 1 mM $Na_3VO_4$, 1 mM β-glycerolphosphate, 1 μg/mL leupeptin, 1X protease inhibitor cocktail, and 1 mM phenylmethylsulfonyl fluoride) and incubated on ice for 10 mins. The lysates were sonicated and kept on ice. The samples were centrifuged at 14,000 × g at 4°C for 10 minutes. Supernatant was collected and stored at −80°C until further use.

### Preparation of Standard Curve

Recombinant human Bcl10 was diluted with Reagent Diluent Buffer (20 mM Trizma, 150 mM NaCl, 0.1% BSA and 0.05% Tween 20, pH 7.4) to a

concentration of 16 ng/mL. Six additional standards were prepared from baseline 16 ng/ml by serial dilutions (8, 4, 2, 1, 0.5, 0.25 ng/mL). After preparation of the standards, 100 μL of the standards or 100 μL of the samples (cell extracts) was added in duplicate to wells of the microtiter plate. 100 μL of buffer alone was added to the blank wells. The plate was sealed and incubated at 37°C for 2 hours. At the end of the incubation, the plate was washed three times with Wash Buffer.

### Addition of Second Antibody

The mouse monoclonal antibody to Bcl10 is an IgG1 isotype, able to recognize amino acids 122–168 of Bcl10. Antibody was diluted with Reagent Diluent Buffer to a concentration of 1 μg/mL, and 100 μL was added to each well. The microtiter plate was sealed and incubated for 1.5 hours at 37°C. The wells were washed three times with Wash Buffer.



### Detection by Goat anti-Mouse IgG-HRP

Anti-mouse IgG-HRP conjugate (Santa Cruz) was diluted to 1:3,000. 100 μL of diluted conjugate was added to each well. The microtiter plate was sealed and incubated for one hour at 37°C. Wells were washed three times.

### Addition of Substrate

TMB and $H_2O_2$ solutions (R&D Systems) were combined in equal volumes immediately prior to use, to form the substrate solution for color development. 100 μL of solution was added to each well. Microtiter plate was sealed and incubated for 20 minutes at RT to develop the colorimetric reaction.

### Measurement of Optical Density

The reaction was stopped by addition of 50 μL of stop solution (2N $H_2SO_4$) to each well. Optical density in the wells was read at 450 nm with a reference filter of 570 nm in an ELISA plate reader (SLT, Spectra). The sample values were normalized with total protein content (BCA™ protein assay kit, Pierce, Rockford, IL) and expressed as ng/mg protein.

## Calculation of Concentration

The log of optical density of the standards was plotted against the log concentration of the standards to draw a standard curve. The concentrations of the samples were quantified by comparison with the standard curve.

### Intra-Assay and Inter-Assay Variations

Three control samples were selected with low, medium, or high Bcl10 values. Values of six replicates in a single assay and in six different assays were determined, in order to assess the intra-assay and inter-assay variations.

### Sample Dilution

The effect of matrix dilution on the performance of the Bcl10 ELISA was determined by sample dilution experiments. Three samples, including one high, one medium, and one low, were diluted by 75%, 50%, and 25% (buffer:sample = 75:25, 50:50, and 25:75). The Bcl10 content in these diluted samples, as well as in the undiluted samples, was determined by ELISA.



### Recovery of Exogenously Added Bcl10 (Spiked Samples)

To determine the precision of the developed ELISA, recovery of exogenously added Bcl10 (spike recovery) was performed by mixing known Bcl10 standards (0.5 ng/mL to 4 ng/mL) with two unknown samples (high and low). All samples were assayed by the Bcl10 ELISA.

### Measurements of Serial Dilutions by ELISA and Comparison with Western Blot

A cell lysate sample was diluted 1:2, 1:4, and 1:8 in Reagent Dilution Buffer. Bcl10 content in the diluted and undiluted samples was determined by both ELISA and Western Blot. Proteins in the whole cell lysates were separated by SDS-PAGE on a 12% gel, then transferred to a nitrocellulose membrane (Amersham Biosciences, Piscataway, NJ) and probed with a Bcl10 antibody (Santa Cruz). Immunoreactive bands were visualized using the ECL detection kit (Amersham).

### Correlation Between Western Blot and Developed Bcl10 ELISA

NCM460 cells in culture were treated with Lambda-Carrageenan (LCG) (1 μg/mL) for 24 hours. At the end of the treatment, the cells were washed three times with PBS and then harvested by scraping. Control or treated cells were lysed in lysate buffer, and then the cell extracts were examined for Bcl10 by either the developed ELISA or by Western blot, as described above. $\beta$-actin band density was used as control. Immunoreactive bands were visualized on X-ray film (Bio-Rad Laboratories, Hercules, CA) using the ECL detection kit (Amersham). Densitometric analysis of the band

intensity from three separate Western blots was compared to the results of the ELISA determinations.

### Measurement of Bcl10 in Stimulated Cells

When the NCM460 cells reached 50–60% confluency, they were treated with a known stimulator of Bcl10, λ-Carrageenan (LCG).[15] The cells were treated with 0.1, 1, or 10 μg/mL of LCG for 24 hours. After treatment, the cells were harvested by scraping and cell lysates prepared, as described above. Bcl10 content in the cell lysates was measured by the ELISA.

### Statistical Analysis

Data presented are the mean ± Standard Deviation (SD). Intra- and -inter-assay coefficients of variation were analyzed for known standards, as well as for unknown, quality control samples. At least three biological samples and two technical replicates were performed for each sample. Statistical significance was determined by one-way ANOVA followed by a post-hoc Tukey's test using GraphPad InStat Software (GraphPad Software, San Diego, CA). A p-value of $<0.05$ is considered statistically significant. Pearson correlation coefficients were calculated using the same software.



## RESULTS AND DISCUSSION

### Composite Standard Curve

The composite standard curve of six different Bcl10 ELISAs conducted on different days is presented in Figure 1. The assay range was 0.25 ng/mL minimum to 16 ng/mL maximum. The slope and y-intercept of the curve were 0.5 ($0.498 \pm 0.039$) and $-0.126$, respectively. The composite standard curve has a sensitivity of 0.25 ng/mL of recombinant human Bcl10 within the 95% confidence limit.

### Intra-Assay and Inter-Assay Variation of Standards

Intra-and inter-assay variations of known standards were calculated by analyzing the optical densities (ODs) of 0.5 ng/mL (low), 2.0 ng/mL (medium) and 8 ng/mL (high) standards of six replicates in the same assay (intra-assay) and in six different assays (inter-assay). Tables 1 and 2 present the intra-and inter-assay variation using the known standards ($n = 6$). Intra-assay coefficient of variation ranged from 2.02% to 4.99%, and inter-assay coefficient of variation was 5.62% to 9.18%.

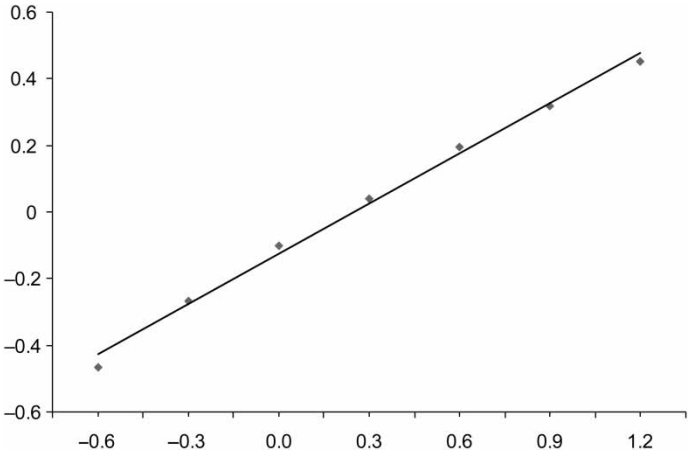



***Figure 1.*** Composite standard curve of Bcl10 ELISA. Composite standard curve of Bcl10 ELISA plots log of Bcl10 concentration vs. log of optical density. Slope is 0.5 and y-intercept is 0.126 (n = 6).



## Intra-Assay and Inter-Assay Variation of Test Samples (QC Samples)

Intra- and inter-assay variations of the low, medium and high sample values were quantified by the developed ELISA (Tables 3 and 4; n = 6). The

***Table 1.*** Intra-assay variation of standards

| | Low standard (0.5 ng/mL) | Medium standard (2 ng/mL) | High standard (8 ng/mL) |
|---|---|---|---|
| Mean (OD) | 0.647 | 1.173 | 2.253 |
| SD | 0.035 | 0.050 | 0.046 |
| CV (%) | 5.384 | 4.297 | 2.052 |

OD = optical density; SD = standard deviation; CV = coefficient of variation.

***Table 2.*** Inter-assay variation of standards

| | Low standard (0.5 ng/mL) | Medium standard (2 ng/mL) | High standard (8 ng/mL) |
|---|---|---|---|
| Mean (OD) | 0.617 | 1.177 | 2.161 |
| SD | 0.057 | 0.104 | 0.121 |
| CV (%) | 9.179 | 8.836 | 5.617 |

OD = optical density; SD = standard deviation; CV = coefficient of variation.

*Table 3.* Intra-assay variation of samples

| | Low QC | Medium QC | High QC |
|---|---|---|---|
| Mean (ng/mL) | 0.535 | 1.884 | 5.355 |
| SD | 0.027 | 0.108 | 0.177 |
| CV (%) | 5.169 | 5.735 | 3.314 |

QC = quality control samples; SD = standard deviation; CV = coefficient of variation.

*Table 4.* Inter-assay variation of samples

| | Low QC | Medium QC | High QC |
|---|---|---|---|
| Mean (ng/mL) | 0.59 | 1.986 | 5.771 |
| SD | 0.057 | 0.145 | 0.364 |
| CV (%) | 9.727 | 7.296 | 6.314 |

QC = quality control samples; SD = standard deviation; CV = coefficient of variation.



intra-assay coefficient of variation ranged from 3.31% to 5.74% and inter-assay coefficient of variation ranged from 6.31% to 9.73%. The low coefficients of variation (<10%) reflect the precision and reproducibility of the developed ELISA.

## Recoveries Following Sample Dilution

Three cell extract samples were diluted 75%, 50%, and 25% with Reagent Diluent Buffer. Bcl10 content was measured by the ELISA. Table 5 presents the tabulated results of these three samples and their dilutions. Undiluted sample values, as determined in the assay, were used to establish the expected values for subsequent dilutions. Recoveries were calculated as the measured concentration divided by the expected concentration and expressed as percentages. Dilution recoveries ranged from 105% to 110%. All results are statistically significant (two-tailed p-values). Pearson's correlation coefficients (r) are: 1.000 (high, $p < 0.001$), 0.9999 (medium, $p = 0.0102$, and 0.998) (low, $p = 0.013$).

## Recovery of Added Exogenous Recombinant Bcl10 ("Spike" Samples)

Two cell extract samples were tested following addition of 0.5 ng, 1 ng, 2 ng, and 4 ng of exogenous, recombinant Bcl10 standard. Control "unspiked"

***Table 5.*** Bcl10 assay results by ELISA following dilution of known sample values

| Sample value (ng/mL) | Dilution sample: buffer | Expected value (ng/mL) | Measured value (ng/mL) | Recovery (%) |
|---|---|---|---|---|
| 7.148 (high) | 100:0 | | 7.148 ± 0.084 | 100 |
| | 75:25 | 5.361 | 5.611 ± 0.248 | 104.676 ± 4.623 |
| | 50:50 | 3.574 | 3.794 ± 0.033 | 106.153 ± 0.925 |
| | 25:75 | 1.787 | 1.967 ± 0.028 | 110.095 ± 1.566 |
| 3.879 (medium) | 100:0 | | 3.879 ± 0.134 | 100 |
| | 75:25 | 2.909 | 3.05 ± 0.034 | 104.851 ± 1.175 |
| | 50:50 | 1.939 | 2.069 ± 0.082 | 106.685 ± 4.264 |
| | 25:75 | 0.97 | 1.033 ± 0.070 | 106.573 ± 7.277 |
| 1.976 (low) | 100:0 | | 1.976 ± 0.034 | 100 |
| | 75:25 | 1.482 | 1.587 ± 0.066 | 107.087 ± 4.488 |
| | 50:50 | 0.988 | 1.082 ± 0.023 | 109.655 ± 2.313 |
| | 25:75 | 0.494 | 0.539 ± 0.035 | 109.172 ± 7.016 |

Pearson's correlation coefficients betweem expected ad measured values are: 1.000 (high, $p < 0.001$), 0.999 (medium, $p = 0.0102$), and 0.998 (low, $p = 0.013$).



samples received an equal volume of Reagent Diluent Buffer. The control sample values, as measured by the assay, were added to the known amount of standard added, in order to establish the expected values for the test "spiked" samples. Recovery was calculated as the measured concentration divided by the expected concentration and expressed as a percentage. Recoveries ranged from 101% to 109% (Table 6). Results are statistically

***Table 6.*** Recovery of exogenous added Bcl10 ("Spike Recovery") to cell extracts

| Sample value (ng/mL) | Spiked value (ng/mL) | Expected value (ng/mL) | Measured value (ng/mL) | Recovery (%) |
|---|---|---|---|---|
| 2.121 ± 0.184 | 0.5 | 2.621 | 2.677 ± 0.059 | 108.084 ± 7.120 |
| (high) | 1.0 | 3.121 | 3.250 ± 0.108 | 109.027 ± 4.238 |
| | 2.0 | 4.121 | 4.073 ± 0.086 | 102.356 ± 4.728 |
| | 4.0 | 6.121 | 6.062 ± 0.282 | 101.393 ± 6.172 |
| 0.547 ± 0.007 | 0.5 | 1.047 | 1.105 ± 0.021 | 105.592 ± 2.04 |
| (low) | 1.0 | 1.547 | 1.621 ± 0.036 | 104.79 ± 2.35 |
| | 2.0 | 2.547 | 2.732 ± 0.037 | 107.301 ± 1.478 |
| | 4.0 | 4.547 | 4.928 ± 0.114 | 108.389 ± 2.495 |

Results are statistically significant. Pearson's correlation coefficients between expected and measured are 0.9993 (high, $p < 0.0007$, two-tailed) and 1.000 (low, $p < 0.0001$, two-tailed).

significant. Correlation coefficients (r) are: 0.9993 (high, 95% C.I. = 0.9635–1.000, $p < 0.0007$) and 1.0000 (low, 95% C.I. = 0.9985–1.000, $p < 0.0001$).

## Serial Dilutions of Cell Samples and Comparison to Western Blot

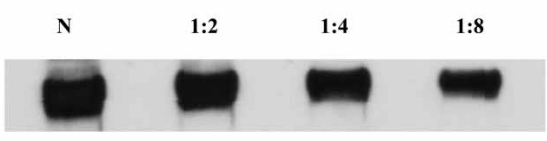
Cell lysate with high Bcl10 content was serially diluted 1:2, 1:4, and 1:8. Bcl10 content was then assessed in the diluted and undiluted samples by either Western blot or ELISA (Figure 2). Width and density of Bcl10 bands in the Western blot were reduced at the higher dilutions (Panel A). Similarly, the ELISA test values declined at higher dilutions (Panel B). The correlation coefficient (r) between the expected and measured ELISA results is 0.9983 ($p = 0.037$, two-tailed t-test).



## Correlation Between Western Blot and Developed ELISA

NCM460 cells grown in 12-well plates were treated with λ-carrageenan (LCG) for 6 hours (1 μg/mL). Control or treated cells were lysed in lysate buffer, and the cell extracts were assayed for Bcl10 by Western Blot or by the solid-phase sandwich ELISA (Figure 3). By Western blot

A




B

| Sample | Values (ng/ml) | Results following dilution (ng/ml) | % Recovery |
|---|---|---|---|
| Undiluted | 7.75 | 7.75 | 100 |
| 1:2 | 3.87 | 4.17±0.25 | 107.81±6.40 |
| 1:4 | 1.94 | 1.94±0.19 | 100.34±9.72 |
| 1:8 | 0.97 | 1.07±0.05 | 111.07±5.00 |

***Figure 2.*** Comparison of Bcl10 by Western blot and ELISA following serial dilutions. Panel A is a Western blot of serial dilutions of a Bcl10 sample. Panel B indicates the results of Bcl10 Elisa and compares the expected and experimental results.

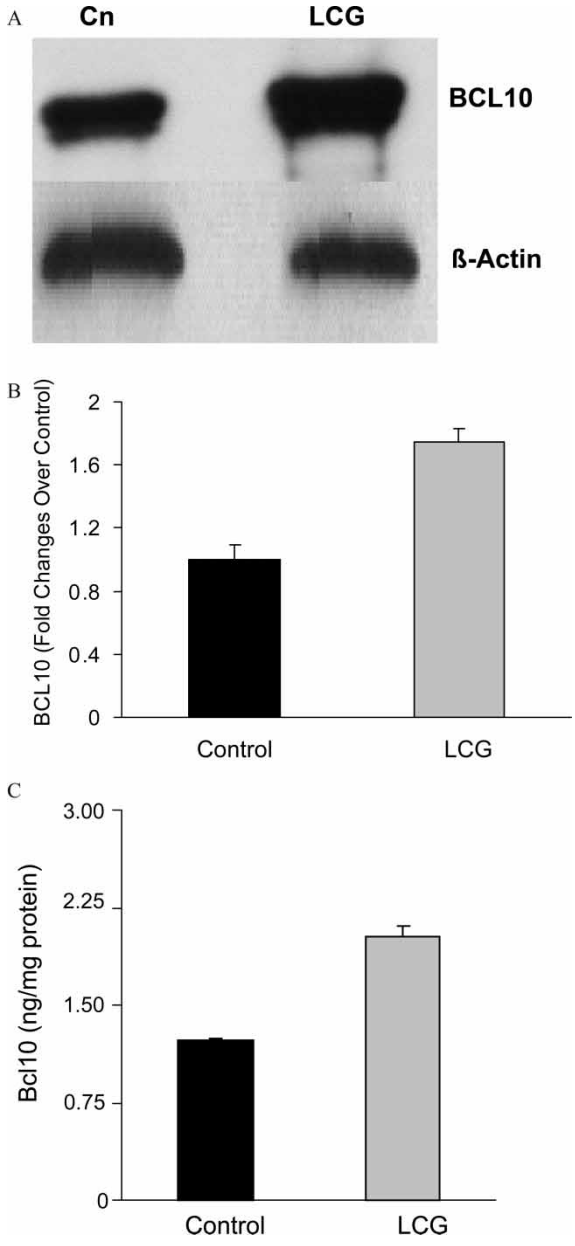



***Figure 3.*** Comparison between Western blot and ELISA for Bcl10 following stimulation. Panel A is the representative Western blot of Bcl10 in NCM460 cells. Lane 1 is unstimulated, lane 2 is stimulated with LCG 1 $\mu$g/mL for 24 hours. Panel B presents the results of densitometry of three Western blots of Bcl10 following stimulation and correction for $\beta$-actin. Panel C presents the corresponding quantitative determinations of Bcl10 by ELISA.

(Panel A), the Bcl10 in treated cells increased 1.75 (LCG) times over control (Panel B). Results of three separate experiments were compared to $\beta$-actin and analyzed by densitometry. By ELISA (Panel C), the Bcl10 values increased from control value of 1.22 ng/mg protein to 2.03 ng/mg protein following LCG, a 1.67-fold increase. Correlation coefficient (r) was 0.9972.

## ELISA Results for Cell Samples Treated with Varying Concentrations of λ-Carrageenan (LCG)

NCM460 cells were exposed to LCG of varying concentrations (0.1μg/mL, 1μg/mL, and 10μg/mL for 24 hours), and Bcl10 values measured. From baseline value of 1.29 $\pm$ 0.09, Bcl10 increased sequentially to 2.80 $\pm$ 0.18, 3.93 $\pm$ 0.37, and 6.36 $\pm$ 0.56 (Figure 4).

## Western Blot and ELISA for Multiple Epithelial Cell Lines

Bcl10 content of several different cell lines was determined by Western blot (Figure 5, Panel A), using a mouse monoclonal antibody raised against amino acids 1-197 of BcL10 of human origin. These results were compared to the Bcl10 assay results by ELISA (Panel B). These determinations identify the presence of Bcl10 in a variety of other epithelial cells, including lung cells and mammary cells.



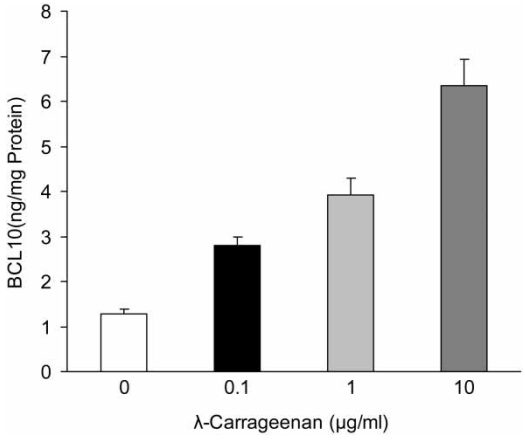



***Figure 4.*** ELISA results for samples treated with increasing concentrations of LCG. Samples were treated with a 100-fold range of LCG from 0.1 μg/mL to 10 μg/mL, with corresponding increases in Bcl10 values from 2.80 $\pm$ 0.18 to 6.36 $\pm$ 0.56 ng/mg protein.

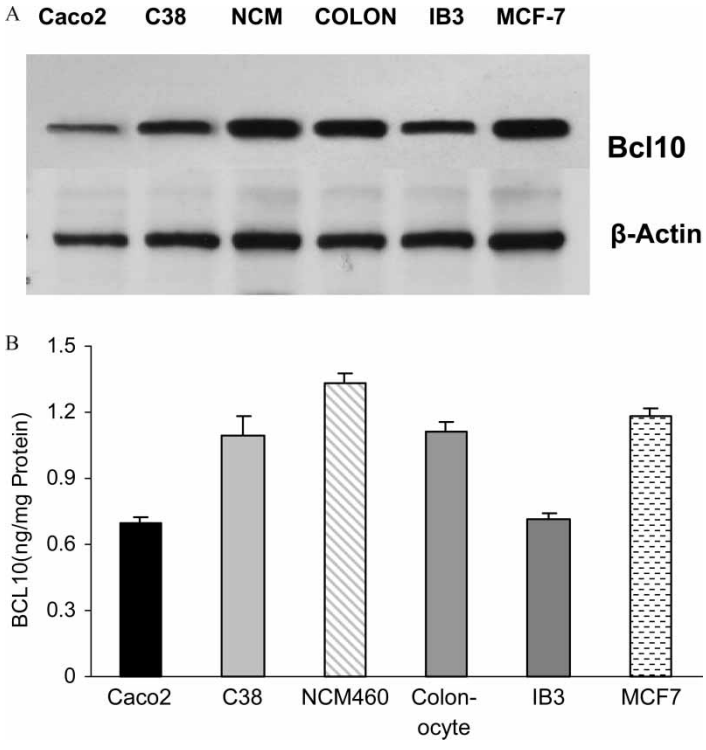



***Figure 5.*** Western blot and ELISA for Bcl10 in multiple epithelial cell lines. Panel A is Western blot of Bcl10 from several different cell lines, including Caco2, NCM460, normal human colonocytes, IB3 and C38 lung cells, and MCF-7 cells. Panel B indicates the results of Bcl10 by Elisa from these different epithelial cells.

## CONCLUSION

We have developed, characterized, and evaluated a solid-phase sandwich ELISA for human Bcl10. To our knowledge this is the first report of the development of an ELISA or quantitative assay for Bcl10. The standard curve demonstrates that the range of the assay is from 0.25 ng/mL to 16 ng/mL. This 64-fold range enables measurements of stimulated, as well as control, samples without dilution. The sensitivity of the assay is 0.25 ng/mL, enabling accurate detection of small quantities of Bcl10. It is likely that this ELISA will have clinical utility, since increased Bcl10 is associated with malignancies of both immune cells, as well as non-immune cells.[3,7,10–13,20,21]

The ELISA utilizes two distinct antibodies, that recognize two different epitopes (5–19 amino acids and 122–168 amino acids) of the Bcl10 protein, enhancing the specificity of the assay. BLAST search of sequence homology indicated no cross-reactivity with other members of the Bcl10 family.

The evaluation of the assay has included examination of reproducibility, intra-assay variation, inter-assay variation, dilution and spiking experiments with known amounts of recombinant Bcl10, and comparison with Western blot of stimulated and unstimulated samples. These measurements have consistently shown that the developed ELISA provides reproducible, precise measurements. The intra- and inter-assay variations of the measured quality control samples reveal $\leq 10\%$ variation in all cases. The accuracy and reproducibility of the assay are further indicated by the dilution and spike recovery experiments. It is apparent from sample dilution assays that matrix dilution has no significant effect on the Bcl10 measurements. Diluted samples showed 4 to 9% positive bias, and spike recovery experiments had low error range. The average difference between expected and measured Bcl10 values ranged from 1.39 to 9.03%, reflecting that the assay recognizes both native Bcl10 and recombinant Bcl10 with similar precision.

The developed ELISA was further evaluated by comparisons with conventional Western Blot that used a third Bcl10 antibody. By densitometry, Western blot demonstrated 1.75-fold increase in Bcl10 over controls when cells were treated with LCG. By ELISA, the Bcl10 increase was similar, 1.67-fold with a correlation ($R^2$) of 0.994. Dose-dependent changes in Bcl10 by LCG were clearly evident by the Bcl10 ELISA. The sensitive and specific, solid-phase, sandwich ELISA for Bcl10, as described in this report, is very well-suited for the accurate determination of Bcl10 values in different experimental conditions, in immune and non-immune cells, and may be useful in a clinical context.



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