DuoSet® IC

Human Bcl-2

Catalog Number DYC827-2
DYC827-5

For the development of sandwich ELISAs to measure natural and recombinant human Bcl-2 in cell lysates.

This package insert must be read in its entirety before using this product.

FOR RESEARCH USE ONLY.
NOT FOR USE IN DIAGNOSTIC PROCEDURES.

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PRINCIPLE OF THE ASSAY

This DuoSet® IC assay kit contains the basic components required for the development of sandwich ELISAs to measure natural and recombinant human Bcl-2 in cell lysates. An immobilized capture antibody binds Bcl-2 present in samples or standards. A biotinylated detection antibody is added and binds to the captured Bcl-2. Streptavidin-HRP is added and binds to the biotinylated detection antibody. A substrate solution is added and color develops in direct proportion to the amount of Bcl-2 bound in the initial step. Color development is stopped and measured using a colorimetric plate reader.

MATERIALS PROVIDED

Store the unopened kit at 2 - 8° C. Do not use past kit expiration date.

			Vials Provided	
Description	**Part #**	**Storage Conditions**	**Cat. # DYC827-2**	**Cat. # DYC827-5**
Bcl-2 Capture Antibody	840764	2 - 8° C	1	2
Bcl-2 Detection Antibody	840765	2 - 8° C	1	2
Bcl-2 Standard	840766	2 - 8° C	1	2
Streptavidin-HRP	890803	2 - 8° C	1	1

DYC827-2 contains sufficient materials to run ELISAs on at least two 96 well plates.*

DYC827-5 contains sufficient materials to run ELISAs on at least five 96 well plates.*

*Provided the following conditions are met:

- The reagents are prepared as described in this package insert.
- The assay is run as described in the General ELISA Protocol on page 5.
- The recommended microplates, buffers, diluents, substrates, and solutions are used.

OTHER MATERIALS REQUIRED

- Aprotinin (Sigma # A6279)
- Leupeptin (Sigma # L8511)
- Pepstatin (Sigma # P4265)
- Phenylmethylsulfonylfluoride (PMSF) (Sigma # P7626)
- Pipettes and pipette tips
- Deionized or distilled water
- 96 well microplates [Costar EIA Plate (Cat. # 2592) is recommended]
- Plate sealers
- Multi-channel pipette, squirt bottle, manifold dispenser, or automated microplate washer

SOLUTIONS REQUIRED

PBS - 137 mM NaCl, 2.7 mM KCl, 8.1 mM Na_2HPO_4, 1.5 mM KH_2PO_4, pH 7.2 - 7.4, 0.2 μm filtered.

Wash Buffer - 0.05% Tween® 20 in PBS, pH 7.2 - 7.4.

Block Buffer - 1% BSA*, 5% Sucrose in PBS, pH 7.2 - 7.4, with 0.05% NaN_3.

IC Diluent #1 - 1% BSA* in PBS, pH 7.2 - 7.4, 0.2 μm filtered.

IC Diluent #4 - 1 mM EDTA, 0.005% Tween 20, 0.5% Triton X-100 in PBS, pH 7.2 - 7.4.

Lysis Buffer #1 - 1 mM EDTA, 0.005% Tween 20, 0.5% Triton X-100, 25 μg/mL Leupeptin, 25 μg/mL Pepstatin, 100 μM PMSF, 3 μg/mL Aprotinin in PBS, pH 7.2 - 7.4.

Substrate Solution - 1:1 mixture of Color Reagent A (H_20_2) and Color Reagent B (Tetramethylbenzidine) (R&D Systems, Cat. # DY999).

Stop Solution - 2 N H_2SO_4.

REAGENT PREPARATION

Bring all reagents to room temperature before use.

Bcl-2 Capture Antibody (Part 840764) - 720 μg/mL of mouse anti-human Bcl-2 antibody when reconstituted with 200 μL of PBS. After reconstitution, store at 2 - 8° C for up to 30 days or aliquot and store at ≤ -20° C in a manual defrost freezer or at ≤ -70° C for up to 3 months.**

Bcl-2 Detection Antibody (Part 840765) - 18 μg/mL of biotinylated mouse anti-human Bcl-2 antibody when reconstituted with 1.0 mL of IC Diluent #1. After reconstitution, store at 2 - 8° C for up to 30 days or aliquot and store at ≤ -20° C in a manual defrost freezer or at ≤ -70° C for up to 3 months.**

Bcl-2 Standard (Part 840766) - 100 ng/mL of recombinant human Bcl-2 when reconstituted with 500 μL of IC Diluent #4. A seven point standard curve using 2-fold serial dilutions and a high standard of 4000 pg/mL is recommended. After reconstitution, store at 2 - 8° C for up to 30 days or aliquot and store at ≤ -20° C in a manual defrost freezer or at ≤ -70° C for up to 3 months.**

Streptavidin-HRP (Part 890803) - 1.0 mL of streptavidin conjugated to horseradish-peroxidase. Store at 2 - 8° C. **DO NOT FREEZE**.

*The use of Serological Proteins Inc. Bovine Serum Albumin, Fraction V, Protease-free (Cat. # 82-045) or Sigma Bovine Serum Albumin (Cat. # A7030) is recommended. All buffers containing BSA must be stored at 2 - 8° C.

**Provided this is within the expiration date of the kit.

Tween is a registered trademark of ICI Americas.

PREPARATION OF SAMPLES

Cell lysates - Rinse cells two times with PBS, making sure to remove any remaining PBS after the second rinse. Solubilize cells at 1 x 10^7 cells/mL in Lysis Buffer #1. Vortex lysates briefly and allow to sit at room temperature for 1 hour before use or store at ≤ -20° C in a manual defrost freezer. Before use, centrifuge at 2000 x g for 5 minutes and remove the supernatant for assaying.

PRECAUTIONS

Some components of this kit contain sodium azide which may react with lead and copper plumbing to form explosive metallic azides. Flush with large volumes of water during disposal.

The Stop Solution suggested for use with this kit is an acid solution. Wear eye, hand, face, and clothing protection when using this material.

TECHNICAL HINTS AND LIMITATIONS

- This DuoSet IC should not be used beyond the expiration date on the kit label.
- Individual results may vary due to differences in technique, plasticware and water sources.
- It is important that the diluents selected for reconstitution and for dilution of the standard reflect the environment of the samples being measured. The diluent suggested in this protocol should be suitable for most cell lysates.
- The type of enzyme and substrate and the concentrations of capture/detection antibodies used can be varied to create an immunoassay with a different sensitivity and dynamic range. A basic understanding of immunoassay development is required for the successful use of these reagents in immunoassays.
- A thorough and consistent wash technique is essential for proper assay performance. Wash Buffer should be dispensed forcefully and removed completely from the wells by aspiration or decanting. Remove any remaining Wash Buffer by inverting the plate and blotting it against clean paper towels.
- Use a fresh reagent reservoir and pipette tips for each step.
- It is recommended that all standards and samples be assayed in duplicate.
- Avoid microbial contamination of reagents and buffers. This may interfere with the sensitivity of the assay. Buffers containing protein should be made under sterile conditions and stored at 2 - 8° C or be prepared fresh daily.

GENERAL ELISA PROTOCOL

A plate layout is provided to record standards and samples assayed.

Plate Preparation

1. Dilute the Capture Antibody to a working concentration of 4 μg/mL in PBS without carrier protein. Immediately coat a 96 well microplate with 100 μL per well of the diluted Capture Antibody. Seal the plate and incubate overnight at room temperature.
2. Aspirate each well and wash with Wash Buffer, repeating the process two times for a total of 3 washes. Wash by filling each well with Wash Buffer (400 μL) using a squirt bottle, multi-channel pipette, manifold dispenser or autowasher. Complete removal of liquid at each step is essential for good performance. After the last wash, remove any remaining Wash Buffer by aspirating or by inverting the plate and blotting it against clean paper towels.
3. Block plates by adding 300 μL of Block Buffer to each well. Incubate at room temperature for 1 - 2 hours.
4. Repeat the aspiration/wash as in step 2. The plates are now ready for sample addition. Alternatively, the Block Buffer can be aspirated after step 3 and the plates can be dried under vacuum. When sealed with desiccant, the plates can be stored at 2 - 8° C for at least 2 months.

Assay Procedure

1. Add 100 μL of sample or standards in IC Diluent #4 per well. Cover with a plate sealer and incubate 2 hours at room temperature.

   Note: *A seven point standard curve using 2-fold serial dilutions and a high standard of 4000 ng/mL is recommended.*
2. Repeat the aspiration/wash as in step 2 of Plate Preparation.
3. Dilute the Detection Antibody to a working concentration of 0.5 μg/mL in IC Diluent #1. Add 100 μL of the diluted Detection Antibody to each well. Cover with a new plate sealer and incubate 2 hours at room temperature.
4. Repeat the aspiration/wash as in step 2 of Plate Preparation.
5. Immediately before use, dilute the Streptavidin-HRP to the working concentration specified on the vial label using IC Diluent #1. Add 100 μL of the diluted Streptavidin-HRP to each well. Incubate for 20 minutes at room temperature. Avoid placing the plate in direct light.
6. Repeat the aspiration/wash as in step 2 of the Plate Preparation.
7. Add 100 μL of Substrate Solution to each well. Incubate for 20 minutes at room temperature. Avoid placing the plate in direct light.
8. Add 50 μL of Stop Solution to each well. Gently tap the plate to ensure thorough mixing.
9. Determine the optical density of each well immediately, using a microplate reader set to 450 nm. If wavelength correction is available, set to 540 nm or 570 nm. If wavelength correction is not available, subtract readings at 540 nm or 570 nm from the readings at 450 nm. This subtraction will correct for optical imperfections in the plate. Readings made directly at 450 nm without correction may be higher and less accurate.

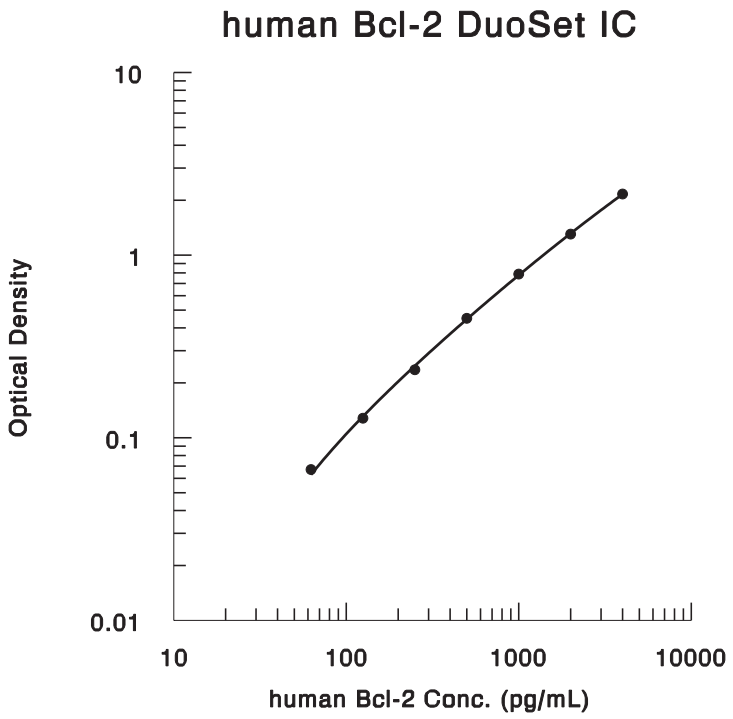
CALCULATION OF RESULTS

Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density.

Create a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the log of the Bcl-2 concentrations versus the log of the O.D. and the best fit line can be determined by regression analysis. This procedure will produce an adequate but less precise fit of the data.

TYPICAL DATA

A standard curve should be generated for each set of samples assayed. The graph below represents typical data generated when using this human Bcl-2 DuoSet IC assay. The standard curve was calculated using a computer generated 4-PL curve-fit. This standard curve is for demonstration purposes only.



CALIBRATION

This DuoSet IC is calibrated against a highly purified *E. coli*-expressed recombinant human Bcl-2 produced at R&D Systems.

SPECIFICITY

The Human Bcl-2 DuoSet IC ELISA is specific for human Bcl-2. Specificity was demonstrated by Western blot analysis of the protein bound by the Capture Antibody supplied in the kit.

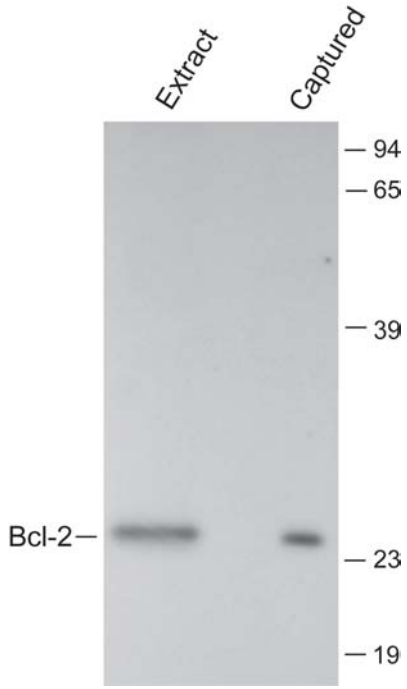


Figure 1: Lysates prepared from human MCF-7 cells were incubated in wells coated with Bcl-2 Duoset IC Capture Antibody. Unbound material was removed by washing and bound material was solubilized in SDS gel sample buffer. Captured proteins were electrophoresed, transferred to an Immobilon (Millipore) membrane and immunoblotted with Bcl-2 Duoset IC Detection Antibody. Only a single band corresponding to human Bcl-2 was detected.

CROSS-REACTIVITY

The following related proteins prepared at 50 ng/mL were assayed in this DuoSet IC and exhibited no cross-reactivity or interference.

rhAI	rhBcl-w	rhBcl-x_L	rhBID

QUANTIFICATION

Absolute amounts of human Bcl-2, as measured by this DuoSet IC, are consistent with the amounts of human Bcl-2 determined by qualitative Western blot analysis.

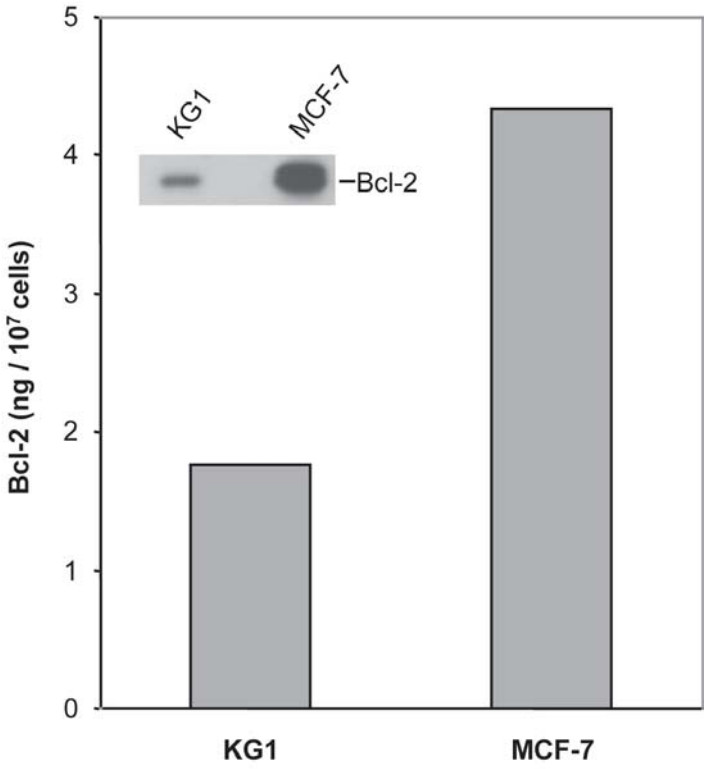
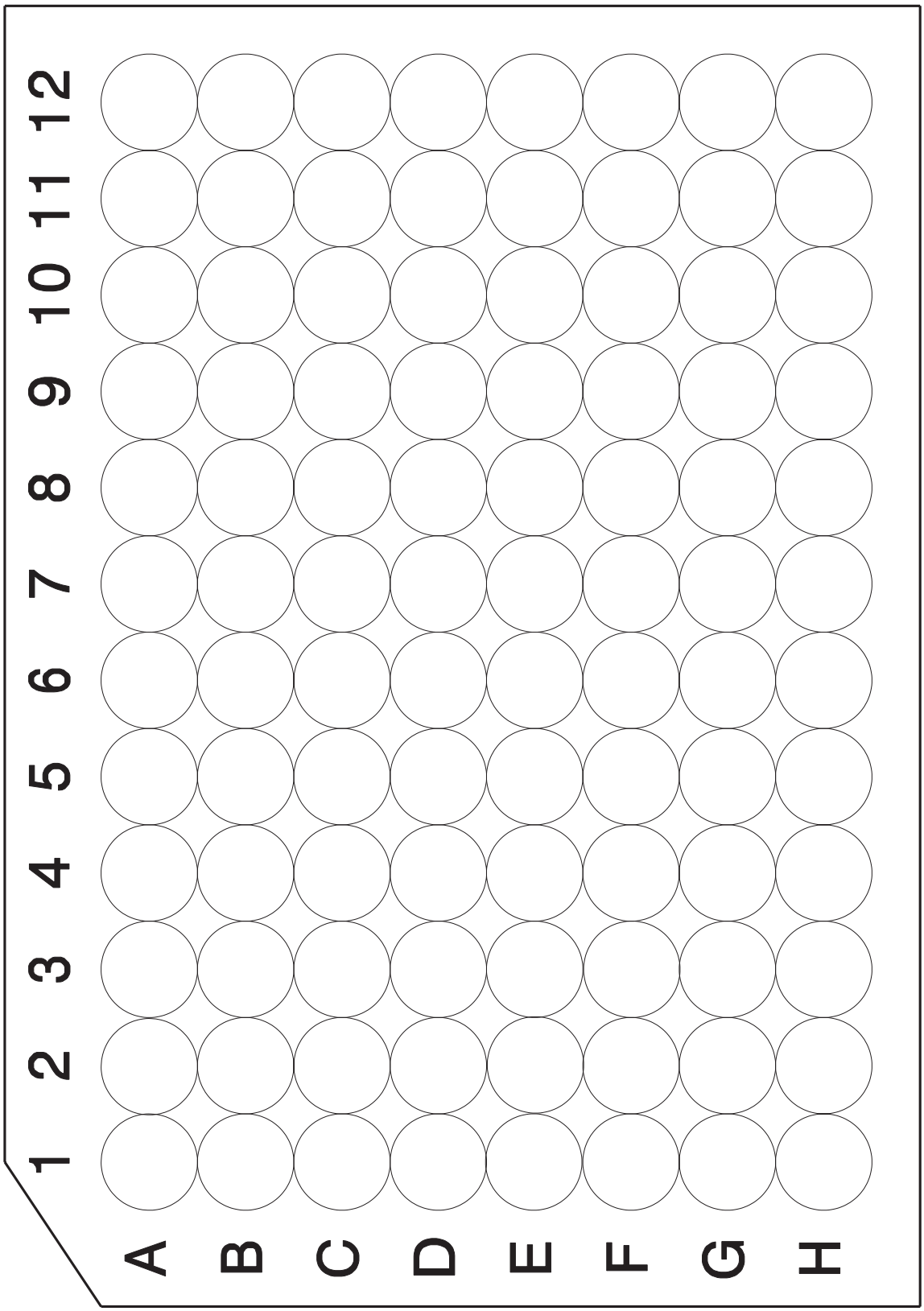


Figure 2: Human KG1 and MCF-7 cells were lysed as described in the Preparation of Samples section on page 4. Human Bcl-2 was measured with this Duoset IC. The same lysates were immunoblotted (see inset) with mouse anti-human Bcl-2. The Duoset IC results correlate well with Bcl-2 amounts detected by Western blot.

PLATE LAYOUT

Use this plate layout as a record of standards and samples assayed.



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