Performance characteristics, continued

Intra-assay precision

Samples of known β-Catenin concentration were assayed in replicates of 12 to determine precision within an assay.

Parameters	Sample 1	Sample 2	Sample 3
Mean (pg/mL)	19.3	7.8	3.8
SD	1.0	0.3	0.2
%CV	5.4	4.3	3.9

SD = Standard Deviation; CV = Coefficient of Variation

Inter-assay precision

Samples were assayed 36 times in multiple assays to determine precision between assays.

Parameters	Sample 1	Sample 2	Sample 3
Mean (pg/mL)	19.2	7.8	3.7
SD	1.3	0.5	0.2
%CV	6.9	6.1	6.0

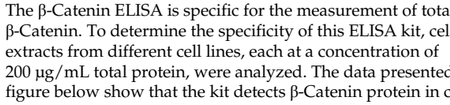
SD = Standard Deviation; CV = Coefficient of Variation

Sensitivity

The minimum detectable dose of β-Catenin is < 0.30 ng/mL. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 32 times.

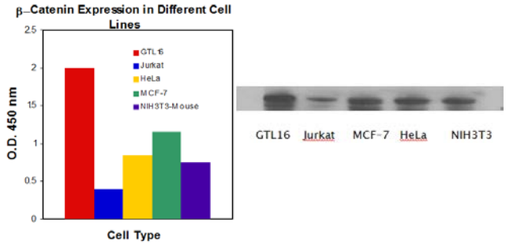
The sensitivity of this ELISA was compared to Western blotting using known quantities of β-Catenin. The data presented below show that the sensitivity of the ELISA is approximately 2× greater than that of Western blotting. The bands shown in the Western blot data were developed using rabbit anti-β-Catenin and an alkaline phosphatase conjugated anti-rabbit IgG followed by chemiluminescent substrate and autoradiography.

Detection of β-Catenin (Total) by ELISA vs Western Blot:



Specificity

The β-Catenin ELISA is specific for the measurement of total β-Catenin. To determine the specificity of this ELISA kit, cell extracts from different cell lines, each at a concentration of 200 µg/mL total protein, were analyzed. The data presented in the figure below show that the kit detects β-Catenin protein in cell lysates from human GTL16, Jurkat, HeLa, MCF-7, and mouse NIH3T3 cells. The levels of β-Catenin protein detected with this ELISA kit are consistent with results obtained by Western blot analysis.



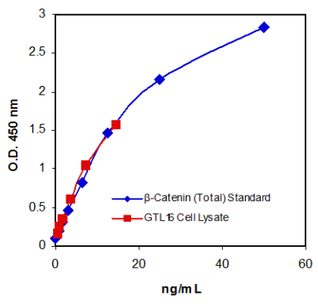
Linearity of dilution

GTL16 cells grown in tissue culture medium containing 10% FBS with Cell Extraction Buffer. This lysate was diluted in Standard Diluent Buffer over the range of the assay and measured for β-Catenin content. Linear regression analysis of samples versus the expected concentration yielded a correlation coefficient of 0.99.

Dilution	Cell Lysate		
	Measured (ng/mL)	Expected (ng/mL)	% Expected
Neat	27.9	27.9	100
1/2	14.4	13.9	103
1/4	8.1	7.0	116
1/8	4.2	3.5	120
1/16	2.2	1.7	124

Parallelism

Natural β-Catenin from GTL16 cell lysates was serially diluted in Standard Diluent Buffer. The optical density of each dilution was plotted against the β-Catenin standard curve. Parallelism was demonstrated by the figure below and indicated that the standard accurately reflects β-Catenin content in samples.



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26 April 2016

β-Catenin (Total) ELISA Kit

Catalog No. KH01211

Pub. No. MAN0014852 **Rev** 2.0

Description

The β-Catenin (Total) ELISA Kit is a solid-phase sandwich Enzyme-Linked Immunosorbent Assay (ELISA) designed to detect and quantify the level of β-Catenin protein independent of its phosphorylation state. The assay is intended for the detection of β-Catenin in lysates of human, mouse, and rat cells.

Contents and storage

The components included in the ELISA kit are listed below. Upon receipt, store the kit at 2°C to 8°C.

Components	Cat. no. KH01211 96 tests
Antibody Coated Wells. 96 well plate.	1 plate
β-Catenin (Total) Detection Antibody. Contains 0.1% sodium azide, blue dye*.	11 mL
β-Catenin (Total) Standard. Lyophilized. Contains 0.1% sodium azide. Refer to vial label for quantity and reconstitution volume.	2 vials
Wash Buffer Concentrate (25X).	100 mL
Standard Diluent Buffer. Contains 0.1% sodium azide, red dye*.	25 mL
Anti-Rabbit IgG HRP (100X). Contains 3.3 mM thymol.	0.125 mL
HRP Diluent. Contains 3.3 mM thymol, yellow dye*.	25 mL
Stabilized Chromogen, Tetramethylbenzidine (TMB).	25 mL
Stop Solution.	25 mL
Adhesive Plate Covers.	3

* To avoid pipetting mistakes, colored Standard Diluent Buffer, Detection Antibody, and HRP Diluent are provided to monitor the addition of solution to each well. Dyes do not interfere with test results.

CAUTION! This kit contains materials with small quantities of sodium azide. Sodium azide reacts with lead and copper plumbing to form explosive metal azides. Upon disposal, flush drains with a large volume of water to prevent azide accumulation. Avoid ingestion and contact with eyes, skin and mucous membranes. In case of contact, rinse affected area with plenty of water. Observe all federal, state, and local regulations for disposal.

Materials required but not provided

- Distilled or deionized water
- Microtiter plate reader with software capable of measurement at or near 450 nm
- Plate washer–automated or manual (squirt bottle, manifold dispenser, or equivalent)
- Calibrated adjustable precision pipettes and glass or plastic tubes for diluting solutions

General Guidelines

- Review the **Procedural guidelines** and **Plate washing directions** in the *ELISA Technical Guide* available at **thermofisher.com** for details prior to starting the procedure.
- Reagents are lot-specific. Do not mix or interchange different reagent lots from various kit lots.

Prepare 1X Wash Buffer

1. Allow the Wash Buffer Concentrate (25X) to reach room temperature and mix to redissolve any precipitated salts.
2. Dilute 16 mL of Wash Buffer Concentrate (25X) with 384 mL of deionized or distilled water. Label as 1X Wash Buffer.
3. Store the concentrate and 1X Wash Buffer in the refrigerator. Use the diluted buffer within 14 days.

For Research Use Only. Not for use in diagnostic procedures.

Manufacturing Site • 7335 Executive Way • Frederick • MD 21704 • E-mail: **techsupport@lifetech.com**

Prepare Cell Extraction Buffer

Cell Extraction Buffer consists of 10 mM Tris (pH 7.4), 100 mM NaCl, 1 mM EDTA, 1 mM EGTA, 1 mM NaF, 20 mM $Na_4P_2O_7$, 2 mM Na_3VO_4, 1% Triton X-100, 10% glycerol, 0.1% SDS , and 0.5% deoxycholate. Add 1 mM PMSF and protease inhibitors just before use.

Refer to the *ELISA Technical Guide* for detailed information on preparing Cell Extraction Buffer.

Prepare cell lysate

1. Collect cells in PBS by centrifugation (non-adherent) or scraping from culture flasks (adherent).
2. Wash cells twice with cold PBS.
3. Remove and discard the supernatant and collect the cell pellet. The pellet can be stored at –80°C and lysed at a later date if desired.
4. Lyse the cell pellet in Cell Extraction Buffer* for 30 minutes, on ice. Vortex at 10 minute intervals.
5. Transfer the lysate into microcentrifuge tubes and centrifuge at 13,000 rpm for 10 minutes at 4°C.
6. Transfer the supernatant into clean microfuge tubes. Samples can be stored at –80°C (avoid multiple freeze-thaw cycles).

* The volume used depends on the number of cells in cell pellet and expression level of β-Catenin. For example, 10^8 GTL16 cells grown in DMEM plus 10% FBS can be extracted in 1 mL of Extraction Buffer. Researchers must optimize the extraction procedures for their own applications.

Dilute samples

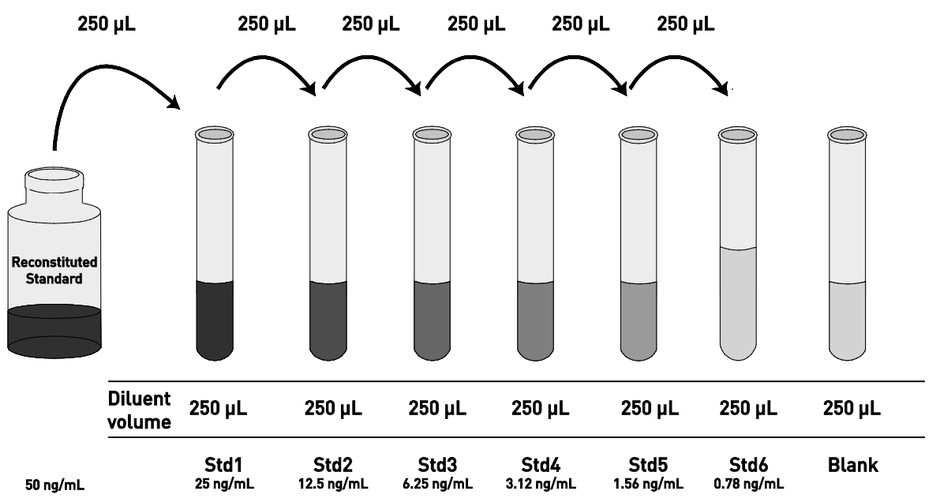
- Dilute samples prepared in Cell Extraction Buffer 10-fold or greater in Standard Diluent Buffer.
- Because conditions may vary, it is recommended that each investigator determine the optimal dilution to be used for each application.

Dilute standards

Note: The β-Catenin (Total) Standard is prepared using recombinant β-Catenin protein.

Note: Use glass or plastic tubes for diluting standards.

1. Reconstitute β-Catenin (Total) Standard to 50 ng/mL with Standard Diluent Buffer. Refer to the standard vial label for instructions. Swirl or mix gently and allow the contents to sit for 10 minutes to ensure complete reconstitution. Label as 50 ng/mL β-Catenin. **Use the standard within 1 hour of reconstitution**.
2. Add 250 µL Standard Diluent Buffer to each of 7 tubes labeled as follows: 25, 12.5, 6.25, 3.12, 1.56, 0.78, and 0 ng/mL β-Catenin.
3. Make serial dilutions of the standard as shown in the dilution diagram below. Mix thoroughly between steps.
4. Discard remaining reconstituted standard or freeze in aliquots at -80°C. Standard can be frozen and thawed one time only without loss of activity. Return Standard Diluent Buffer to the refrigerator.



Prepare 1X Anti-Rabbit IgG HRP solution

Note: Prepare Anti-Rabbit IgG HRP within 15 minutes of usage.

The Anti-Rabbit IgG HRP (100X) is in 50% glycerol, which is viscous. To ensure accurate dilution:

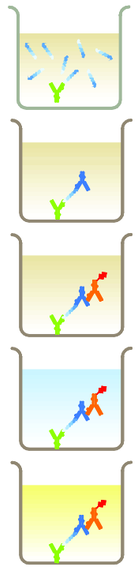
1. For each 8-well strip used in the assay, pipet 10 µL Anti-Rabbit IgG HRP (100X) solution, wipe the pipette tip with a clean absorbent paper to remove any excess solution, and dispense the solution into a tube containing 1 mL of HRP Diluent. Mix thoroughly.
2. Return the unused Anti-Rabbit IgG HRP (100X) solution to the refrigerator.

ELISA procedure

Allow all reagents to reach room temperature before use. Mix all liquid reagents prior to use. **Total assay time is 4 hours.**

IMPORTANT! Perform a standard curve with each assay.

Determine the number of 8-well strips required for the assay. Insert the strips in the frames for use. Re-bag any unused strips and frames, and store at 2 to 8°C for future use.



Bind antigen

1. Add 100 µL Standard Diluent Buffer to zero standard wells. Wells for chromogen blank should be left empty.
2. Add 100 µL of standards and diluted samples or controls (see Dilute samples) to the appropriate wells.
3. Cover the plate with the plate cover and incubate for 2 hours at room temperature.
4. Thoroughly aspirate the solution and wash wells 4 times with 1X Wash Buffer.

Add detector antibody

5. Add 100 µL β-Catenin (Total) Detection Antibody solution into each well except the chromogen blanks.
6. Cover the plate with plate cover and incubate for 1 hour at room temperature.
7. Thoroughly aspirate the solution and wash wells 4 times with 1X Wash Buffer.

Add 1X Anti-Rabbit IgG HRP

8. Add 100 µL 1X Anti-Rabbit IgG HRP (see page 2) into each well except the chromogen blanks.
9. Cover the plate with the plate cover and incubate for 30 minutes at room temperature.
10. Thoroughly aspirate the solution from the wells and wash wells 4 times with 1X Wash Buffer.

Add chromogen

11. Add 100 µL Stabilized Chromogen to each well. The substrate solution will begin to turn blue.
12. Incubate for 30 minutes at room temperature **in the dark**.
    Note: TMB should not touch aluminum foil or other metals.

Add stop solution

13. Add 100 µL of Stop Solution to each well. Tap side of the plate gently to mix. The solution in the wells changes from blue to yellow.

Capture Antibody · Antigen · Detector Antibody · HRP-Conjugate Antibody

Read the plate and generate the standard curve

1. Read the absorbance at 450 nm. Read the plate within 2 hours after adding the Stop Solution.
2. Use curve-fitting software to generate the standard curve. A four parameter algorithm provides the best standard curve fit. Optimally, the background absorbance may be subtracted from all data points, including standards, unknowns and controls, prior to plotting.
3. Read the concentrations for unknown samples and controls from the standard curve. Multiply value(s) obtained for sample(s) by the appropriate factor to correct for the sample dilution.

   Note: Dilute samples producing signals greater than that of the highest standard in Standard Diluent Buffer and reanalyze. Multiply the concentration by the appropriate dilution factor.

Performance characteristics

Standard curve (example)

The following data were obtained for the various standards over the range of 0-50 ng/mL β-Catenin.

Standard β-Catenin (ng/mL)	Optical Density (450 nm)
50	2.84
25	2.17
12.5	1.46
6.25	0.83
3.12	0.47
1.56	0.32
0.78	0.19
0	0.10

Recovery

To evaluate recovery, β-Catenin Standard was spiked at three different concentrations into 15% Cell Extraction Buffer. The percent recovery was calculated as an average of 124%.