PRODUCT INFORMATION

β Catenin EIA kit

Catalog No. IB09614
96 Well Kit

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

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Description

The β-catenin EIA kit is a complete kit for the determination of β-catenin in cell lysates.
Please read the complete kit insert before performing this assay. The kit uses a monoclonal antibody to β-catenin immobilized on a microtiter plate to bind the β-catenin in the standards or sample. A recombinant human β-catenin Standard is provided in the kit. After a short incubation the excess sample or standard is washed out and a polyclonal antibody to β-catenin is added. This antibody binds to the β-catenin captured on the plate. After a short incubation the excess antibody is washed out and Goat anti-Rabbit IgG conjugated to Horseradish peroxidase is added, which binds to the polyclonal β-catenin antibody. Excess conjugate is washed out and substrate is added. After a short incubation, the enzyme reaction is stopped and the color generated is read at 450 nm. The measured optical density is directly proportional to the concentration of β-catenin in either standards or samples. For further explanation of the principles and practices of immunoassays please see the excellent books by Chard[1] or Tijssen[2].

Introduction

β-catenin is a 781 amino acid adherens junction protein that plays an important role in two unrelated cellular functions. Adherens junctions are critical for establishment and maintenance of epithelial layers, such as those found lining the surface of organs. Involved in both cell-cell adhesion and in the wnt/wg signaling pathway, β-catenin can be found in the cytoplasm or the nucleus[3]. In the cytoplasm, β-catenin is unstabilized by phosphorylation and association with E-cadherin. When β-catenin translocates to the nucleus, it has comparatively low levels of phosphorylation. Wnt/wg signaling results in β-catenin accumulation and transcriptional activation of specific target genes during development. Deregulation of β-catenin signaling is an important event in the evolution a number of malignancies, including colon cancer[4], melanoma, ovarian cancer[5], and prostate cancer[6].

The homology between human, mouse and rat β-catenin is 99.9%.

Precautions

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1. Stop Solution 2 is a 1N hydrochloric acid solution. This solution is caustic; care should be taken in use.
2. The activity of the Horseradish peroxidase conjugate is affected by nucleophiles such as azide, cyanide and hydroxylamine.
3. We test this kit's performance with a variety of buffers, however it is possible that high levels of interfering substances may cause variation in assay results.
4. The β-catenin Standard provided, Catalog No. 80-1269, should be handled with care because of the known and unknown effects of β-catenin.
5. The β-catenin standard should be stored at or below -20 °C.
6. Standards and samples must be prepared in polypropylene tubes. Preparation in glass will result in decreased protein stability.

Materials Supplied

1. **β-catenin Microtiter Plate, One Plate of 96 Wells, Catalog No. 80-1235**
   A plate using break-apart strips coated with a mouse monoclonal antibody raised against amino acids 571-781 of mouse β-catenin.
2. **Total β-catenin Antibody, 11 mL, Catalog No. 80-1236**
   A yellow solution of rabbit polyclonal antibody raised against a 15 amino acid synthetic peptide sequence derived from the C-terminus of human β-catenin.
3. **Assay Buffer 13, 1x, 105 mL, Catalog No. 80-1509**
   Tris buffered saline containing proteins and detergents.
4. **Total β-catenin Conjugate, 11 mL, Catalog No. 80-1273**
   A blue solution of Goat anti-Rabbit IgG conjugated to Horseradish peroxidase.
5. **Wash Buffer Concentrate, 105 mL, Catalog No. 80-1287**
   Tris buffered saline containing detergents.
6. **human β-catenin Standard, 1 vial, Catalog No. 80-1269**
   A solution of 80,000 pg/mL recombinant human β-catenin.
7. **TMB Substrate, 12 mL, Catalog No. 80-0350**
   A solution of 3,3',5,5' tetramethylbenzidine (TMB) and hydrogen peroxide. **Protect from prolonged exposure to light.**
8. **Stop Solution 2, 11 mL, Catalog No. 80-0377**
   A 1N solution of hydrochloric acid in water. Keep tightly capped. Caution: **Caustic.**
9. **RIPA Cell Lysis Buffer 2, 105 mL, Catalog No. 80-1284**
   50 mM Tris HCl, pH 7.4, 150 mM NaCl, 1 mM EDTA, 1 mM EGTA, 1% Triton X-100, 1% sodium deoxycholate and 0.1% SDS.
10. **β-catenin Assay Layout Sheet, 1 each, Catalog No. 30-0211**
11. **Plate Sealer, 3 each, Catalog No. 30-0012**

Storage

All components of this kit, **except the β-catenin Standard,** are stable at 4 °C until the kit's expiration date. The β-catenin Standard **must** be stored at or below -20 °C.

Materials Needed but Not Supplied

1. Deionized or distilled water.
2. Phenylmethylsulfonyl fluoride (PMSF), Sigma # P7626 or equivalent.
3. Protease Inhibitor Cocktail (PIC), Sigma # P8340 or equivalent.
4. Precision pipets for volumes between 100 µL and 1,000 µL.
5. Repeater pipet for dispensing 100 µL.
6. Disposable beakers for diluting buffer concentrates.
7. Graduated cylinders.
8. A microplate shaker.
9. Adsorbent paper for blotting.
10. Microplate reader capable of reading at 450 nm, preferably with correction between 570 nm and 590 nm.
11. Graph paper for plotting the standard curve.
12. Polpropylene test tubes.

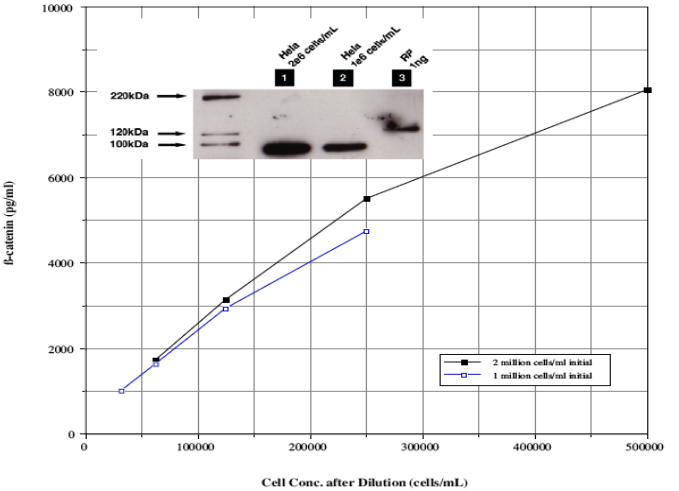
Sample Handling

The β-catenin EIA kit is compatible with β-catenin samples in a wide range of cell lysates and buffers. Samples diluted sufficiently into Assay Buffer 13 plus Inhibitors (see Reagent Preparation, page 5, #2) can be read directly from a standard curve. Please refer to the Sample Recovery recommendations on page 11 for details of suggested dilutions. It is recommended that all samples be lysed with the provided RIPA Cell Lysis Buffer 2 modified by the addition of PIC and PMSF (see Reagent Preparation, page 6, #4) immediately prior to use. Samples lysed in this modified RIPA Cell Lysis Buffer 2 must be diluted at least 1:8 with Assay Buffer 13 plus Inhibitors prior to assaying. Note that this dilution is based on the lysis of 1.0 million HeLa cells per mL. The 1:8 dilution contained 125,000 cells per mL with a calculated recovery of 107.1%.

If the end user chooses to use another lysis buffer, a greater number of cells, or varies from the stimulation procedure noted below, it is up to the end user to determine the appropriate dilution of samples and assay validation. Only standard curves generated in modified Assay Buffer 13 should be used to calculate the concentration of β-catenin. Samples must be stored frozen at or below -70 °C to avoid loss of bioactive β-catenin. Excessive freeze/thaw cycles should be avoided. Prior to assay, frozen samples should be brought to 4 °C slowly and gently mixed. **Samples must be prepared in polypropylene tubes.**

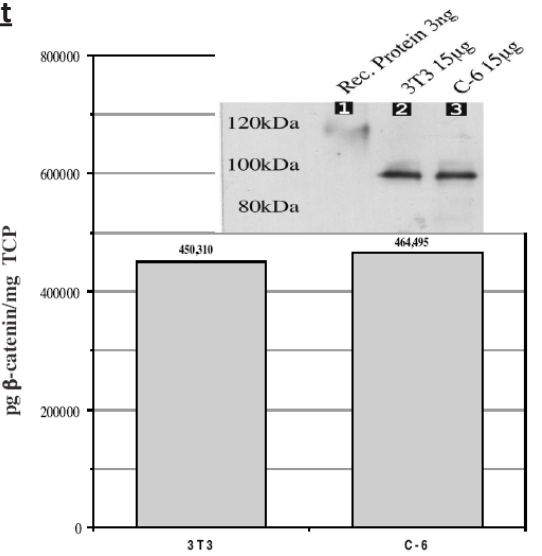
HeLa Cell Lysate Experiment

The number of HeLa cells used in this experiment were 2 million and 1 million per mL. Cells were centrifuged at 1,500 rpm for 5 minutes and the supernatant discarded. The cell pellets were resuspended and washed with PBS. Cells were pelleted at 1,500 rpm for 5 minutes and the supernatant dicarded. The cell pellets were resuspended with modified RIPA Cell Lysis Buffer 2. The lysates were vortexed and centrifuged at 14,000 rpm for 10 minutes. The supernatants were split for the Western blot and EIA to generate the data illustrated. Note that 24 µL of sample was used per lane in the Western blot. The exposure time for development was 30 minutes.



Mouse and Rat Cell Lysate Experiment

Mouse NIH 3T3 and rat C6 cells were harvested, centrifuged at 1,500 rpm for 5 minutes and the supernatants discarded. The cell pellets were washed four times with PBS and then resuspended with modified RIPA Cell Lysis Buffer 2. The lysates were placed on ice for 30 minutes and then centrifuged at 16,000 rpm for 20 minutes at 4 °C. The supernatants were stored at -80 °C prior to application in Western blot and EIA to generate the data illustrated. It is recommended no more than 0.025 mg/mL of total cellular protein be used in the assay.



Procedural Notes

1. Do not mix components from different kit lots or use reagents beyond the kit expiration date.
2. Allow all reagents to warm to room temperature for at least 30 minutes before opening.
3. **Standards and samples must be made up in plastic tubes.**
4. Pre-rinse the pipet tip with reagent, use fresh pipet tips for each sample, standard and reagent.
5. Pipet standards and samples to the bottom of the wells.
6. Add the reagents to the side of the well to avoid contamination.
7. This kit uses break-apart microtiter strips, which allow the user to measure as many samples as desired. Unused wells must be kept desiccated at 4 °C in the sealed bag provided. The wells should be used in the frame provided.
8. **Prior to addition of antibody, conjugate and substrate, ensure that there is no residual wash buffer in the wells. Any remaining wash buffer may cause variation in assay results.**

Reagent Preparation

1. **Wash Buffer**
   Prepare the Wash Buffer by diluting 50 mL of the supplied concentrate with 950 mL of deionized water. This can be stored at room temperature until the kit expiration, or for 3 months, whichever is earlier.

2. **Assay Buffer 13 plus Inhibitors**
   Immediately prior to use in the assay, PMSF and PIC must be added to the buffer. If using Sigma Protease Inhibitor Cocktail #P8340, add 0.5 µL/mL PIC or equivalent concentration according to alternate vendor's specification sheet. Add PMSF, such as Sigma #P7626, to a final concentration of 1 mM.
   This modified Assay Buffer 13 must be used for all sample and standard dilutions to ensure optimal integrity of β-catenin. Fresh Assay Buffer 13 plus Inhibitors must be made for each assay.

3. **human β-catenin Standards**
   Allow the human β-catenin standard solution to warm to room temperature. Label seven polypropylene tubes #1 through #7. Pipet 900 µL of Assay Buffer 13 plus Inhibitors into tube #1. Pipet 500 µL of Assay Buffer 13 plus Inhibitors into tubes #2 through #7. Add 100 µL of the 80,000 pg/mL standard into tube #1 and vortex. Add 500 µL of tube #1 to tube #2 and vortex thoroughly. Add 500 µL of tube #2 to tube #3 and vortex thoroughly. Continue this for tubes #4 through #7.

   The concentration of β-catenin in standards #1 through #7 will be 8,000, 4,000, 2,000, 1,000, 500, 250 and 125 pg/mL respectively. See β-catenin Assay Layout Sheet for dilution details.

   Diluted standards should be used within 60 minutes of preparation.

   Discard any unused standard dilutions.

4. **RIPA Cell Lysis Buffer 2**
   Allow to come to room temperature. Ensure buffer is completely in solution prior to use. Immediately prior to use for cell lysis, PMSF and PIC must be added to the buffer. If using Sigma Protease Inhibitor Cocktail #P8340, add 0.5 µL/mL PIC or equivalent concentration according to alternate vendor's specification sheet. Add PMSF, such as Sigma #P7626, to a final concentration of 1 mM.

Assay Procedure

Bring all reagents to room temperature for at least 30 minutes prior to opening.

All standards, controls and samples should be run in duplicate.

1. Refer to the Assay Layout Sheet to determine the number of wells to be used and put any remaining wells with the desiccant back into the pouch and seal the ziploc. Store unused wells at 4 °C.
2. Pipet 100 µL of Assay Buffer 13 plus Inhibitors into the S0 (0 pg/mL standard) wells.
3. Pipet 100 µL of Standards #1 through #7 into the appropriate wells.
4. Pipet 100 µL of the Samples into the appropriate wells.
5. Tap the plate gently to mix the contents.
6. Seal the plate and incubate at room temperature on a plate shaker for 1 hour at ~500 rpm.
7. Empty the contents of the wells and wash by adding 400 µL of wash solution to every well. Repeat the wash 4 more times for a total of **5 washes**. After the final wash, empty or aspirate the wells and firmly tap the plate on a lint free paper towel to remove any remaining wash buffer.
8. Pipet 100 µL of yellow Antibody into each well, except the Blank.
9. Seal the plate and incubate at room temperature on a plate shaker for 1 hour at ~500 rpm.
10. Empty the contents of the wells and wash by adding 400 µL of wash solution to every well. Repeat the wash 4 more times for a total of **5 washes**. After the final wash, empty or aspirate the wells and firmly tap the plate on a lint free paper towel to remove any remaining wash buffer.
11. Add 100 µL of blue Conjugate to each well, except the Blank.
12. Seal the plate and incubate at room temperature on a plate shaker for 30 minutes at ~500 rpm.
13. Empty the contents of the wells and wash by adding 400 µL of wash solution to every well. Repeat the wash 4 more times for a total of **5 washes**. After the final wash, empty or aspirate the wells and firmly tap the plate on a lint free paper towel to remove any remaining wash buffer.
14. Pipet 100 µL of Substrate Solution into each well.
15. Incubate for 30 minutes at room temperature on a plate shaker at ~500 rpm.
16. Pipet 100 µL Stop Solution 2 to each well.
17. Blank the plate reader against the Blank wells, read the optical density at 450 nm, preferably with correction between 570 and 590 nm. If the plate reader is not able to be blanked against the Blank wells, manually subtract the mean optical density of the Blank wells from all the readings.

Calculation of Results

Several options are available for the calculation of the concentration of β-catenin in the samples. We recommend that the data be handled by an immunoassay software package utilizing a 4 parameter logistic curve fitting program. If data reduction software is not readily available, the concentration of β-catenin can be calculated as follows:

1. Calculate the average net Optical Density (OD) bound for each standard and sample by subtracting the average Blank OD from the average OD for each standard and sample.

   Average Net OD = Average OD - Average Blank OD

2. Using linear graph paper, plot the Average Net OD for each standard versus β-catenin concentration in each standard. Approximate a straight line through the points. The concentration of β-catenin in the unknowns can be determined by interpolation.

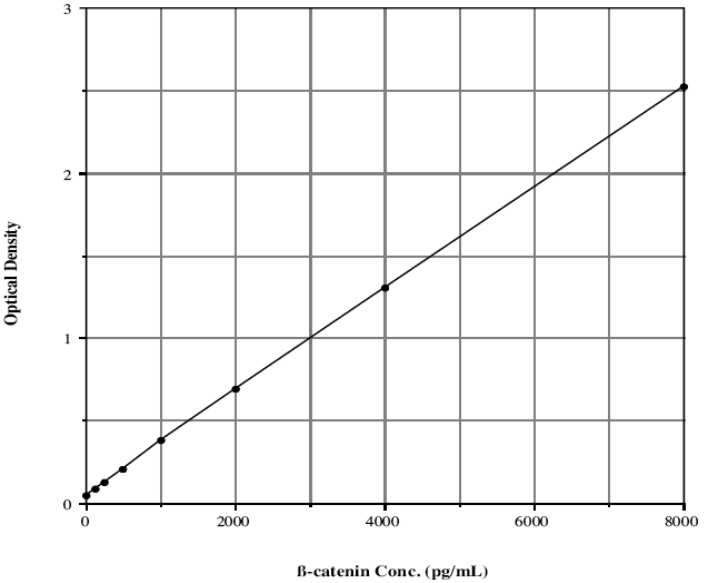
Typical Results

The results shown below are for illustration only and **should not** be used to calculate results from another assay.

Sample	Average OD	Net OD	β-**catenin (pg/mL)**
Blank	(0.064)		
S0	0.113	0.049	**0**
S1	2.589	2.525	**8,000**
S2	1.368	1.304	**4,000**
S3	0.758	0.694	**2,000**
S4	0.449	0.385	**1,000**
S5	0.269	0.205	**500**
S6	0.190	0.126	**250**
S7	0.150	0.086	**125**
Unknown 1	0.487	0.423	**1,163**
Unknown 2	0.313	0.249	**619**

Typical Standard Curve

A typical standard curve is shown below. This curve **must not** be used to calculate β-catenin concentrations; each user must run a standard curve for each assay.



Performance Characteristics

The following parameters for this kit were determined using the guidelines listed in the National Committee for Clinical Laboratory Standards (NCCLS) Evaluation Protocols[7].

Sensitivity

Sensitivity was calculated by determining the average optical density bound for twenty (20) wells run with 0 pg/mL Standard, and comparing to the average optical density for twenty (20) wells run with Standard #7. The detection limit was determined as the concentration of β-catenin measured at two (2) standard deviations from the 0 pg/mL Standard along the standard curve.

Mean OD for S0 = 0.137 ± 0.006 (4.38%)
Mean OD for Standard #7 = 0.182 ± 0.007 (3.85%)
Delta Optical Density (125 - 0 pg/mL) = 0.182 - 0.137 = 0.045

2 SD's of 0 pg/mL Standard = 2 x 0.006 = 0.012

Sensitivity = $\frac{0.012}{0.045}$ x 125 pg/mL = **33.3 pg/mL**

Linearity

A sample containing 6,107 pg/mL β-catenin was serially diluted 5 times 1:2 in the Assay Buffer 13 plus Inhibitors supplied in the kit and measured in the assay. The data was plotted graphically as actual β-catenin concentration versus measured β-catenin concentration.

The line obtained had a slope of 1.01 with a correlation coefficient of 0.999.

Precision

Intra-assay precision was determined by taking samples containing low, medium and high concentrations of β-catenin and running these samples multiple times (n=24) in the same assay. Inter-assay precision was determined by measuring three samples with low, medium and high concentrations of β-catenin in multiple assays (n=10).

The precision numbers listed below represent the percent coefficient of variation for the concentrations of β-catenin determined in these assays as calculated by a 4 parameter logistic curve fitting program.

	β-catenin (pg/mL)	Intra-assay % CV	Inter-assay % CV
Low	541.0	3.8	
Medium	1,041.8	3.7	
High	5,796.4	3.1	
Low	610.9		8.4
Medium	1,141.4		8.8
High	6,041.5		2.9

Cross Reactivities

The cross reactivities for a number of related compounds were determined by dissolving the cross reactant in the kit assay buffer. These samples were then measured in the Total β-catenin assay and the measured β-catenin concentration calculated. The % cross reactivity was calculated by comparison with the actual concentration of cross reactant in the sample and expressed as a percentage.

Compound	Cross Reactivity
human β-catenin	100%
cIAP1	<0.1%
Smac/DIABLO	<0.1%
p300	<0.1%
MEK2	<0.1%
GSK-3β	<0.1%

Sample Recoveries

Please refer to pages 4 and 6 for Sample Handling recommendations and Standard preparation.

β-catenin concentrations were measured in modified RIPA Cell Lysis Buffer 2. β-catenin was spiked into the undiluted sample, which was then diluted with Assay Buffer 13 plus Inhibitors and assayed in the kit. The following result was obtained:

Sample	% Recovery*	Recommended Dilution*
RIPA Cell Lysis Buffer 2	88.0%	≥1:8

For the lysis of 2 million and 1 million HeLa cells per mL, the following results were obtained:

Sample	% Recovery*	Recommended Dilution*
2 million cells per mL	98.8%	≥1:8
1 million cells per mL	107.1%	≥1:8

WARNING: If the end user chooses to not use the provided RIPA Cell Lysis Buffer 2, it is up to the end user to determine the appropriate dilution of samples and assay validation for their chosen cell lysis buffer.

* See Sample Handling instructions on page 4 for details.

References

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5. J.M. Shannon, B.A. Hyatt, Annu. Rev. Physiol., (2004) 66:625-45. Review.
6. P.J. Morin, Bioessays, (1999) 21(12):1021-30. Review.
7. National Committee for Clinical Laboratory Standards Evaluation Protocols, SC1, (1989) Villanova, PA: NCCLS.

Use of Product

This product contains research chemicals. As such, they should be used and handled only by or under the supervision of technically qualified individuals. This product is not intended for diagnostic or human use.

Warranty

IBL America, Inc. makes no warranty of any kind, expressed or implied, which extends beyond the description of the product in this brochure, except that the material will meet our specifications at the time of delivery. IBL-America, Inc. makes no guarantee of results and assumes no liability for injuries, damages or penalties resulting from product use, since the conditions of handling and use are beyond our control.

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