β-catenin (human) ELISA Kit

(Catalog # K3381-100, 100 assays; Store at 4°C)

I. Introduction:

β-catenin (cadherin-associated protein beta, 88 kDa) is a dual function protein, regulating the coordination of cell–cell adhesion and gene transcription via the Wnt signaling pathway. Mutations and overexpression of β-catenin are associated with many cancers, including hepatocellular, colorectal, lung, breast, ovarian and endometrial cancers. BioVision's human β-catenin ELISA Kit is based on the standard principle of a sandwich enzyme-linked immunosorbent assay. Human β-catenin antibody is coated on a 96-well plate. Standards and test samples are added to the wells and β-catenin present in a sample is bound by the immobilized antibody. An HRP-conjugate reagent is added subsequently. After washing away the unbound antibody/HRP conjugates, HRP substrate TMB is used to visualize the HRP enzymatic reaction. TMB is catalyzed by HRP to produce a blue product that changes into yellow after adding acidic stop solution. The density of yellow color is proportional to the human β-catenin captured onto the plate. This ELISA kit shows no species cross-reactivity. Detection Range: 0.156 – 10 ng/ml.

II. Application:

Quantitative detection of β-catenin, establishing normal range etc.

III. Specificity:

Human β-catenin

IV. Sample Type:

- Serum and other biological fluids
- Cell culture medium, tissue or cell lysates

V. Kit Contents:

Components	K3630-100	Part No.
96 wells coated with anti-human β-catenin antibody, 1 Microplate	12 strips x 8 wells	K3381-100-1
Human β-catenin standard (18 ng/ml)	0.5 ml	K3381-100-2
HRP-conjugate reagent	6 ml	K3381-100-3
Standard Diluent	1.5 ml	K3381-100-4
Sample Diluent	6 ml	K3381-100-5
Chromogen Solution A	6 ml	K3381-100-6
Chromogen Solution B	6 ml	K3381-100-7
Stop Solution	6 ml	K3381-100-8
Wash Solution (30x stock)	20 ml	K3381-100-9
Sealed Bag	1	K3381-100-10
Plate cover	2	K3381-100-11

VI. User Supplied Reagents and Equipment:

- Microplate reader capable of measuring absorbance at 450 nm.
- Absorbent paper.
- Adjustable pipettes and pipette tips. Multichannel pipettes are recommended for large number of samples.

VII. Storage Conditions and Reagent Preparation:

Store unopened kit at 4°C for 12 months, protected from light. The antibody-coated microplate must be stored in a dry place at 4°C, sealed in the bag provided. Equilibrate all components to Room Temperature before starting the assay.

- **Preparation of 1x wash solution**: Dilute the 30x concentrated stock 1:30 with distilled water and mix thoroughly. Prepare 0.35 ml of working wash solution for a single wash for each well. The 20 ml stock will make 600 ml of working wash solution.
  Note: If there is precipitation in the wash solution, gently warm to 37°C to dissolve.
- **Human β-catenin Standard Preparation**: Label 6 tubes with 18, 9, 4.5, 2.25, 1.125 and 0.5625 ng/ml of β-catenin Standard. Add 50 µl Standard Diluent to tubes 2-6.

Tube 1: Aliquot 150 µl of the provided Human β-catenin Standard (18 ng/ml)

Tube 2: Add 50 µl from Tube 1 and mix to make 9 ng/ml.

Tube 3: Add 50 µl from Tube 2 and mix to make 4.5 ng/ml.

Tube 4: Add 50 µl from Tube 3 and mix to make 2.25 ng/ml.

Tube 5: Add 50 µl from Tube 4 and mix to make 1.125 ng/ml.

Tube 6: Add 50 µl from Tube 5 and mix to make 0.5625 ng/ml.

Sample Preparation and Storage:

a. Centrifuge cell culture media or biological fluids for 20 mins at 2000-3000 rpm to remove particulates. For serum samples, clot in a serum separator tube (20-30 mins) at room temperature. Centrifuge at approximately 2000-3000 rpm for 20 min and use the supernatant. For cells and tissues, homogenize in PBS (pH 7.2 – 7.4), spin at top-speed in a table-top centrifuge and collect supernatant.
   Notes:
b. For all samples, aliquot and freeze samples at -80°C. Avoid repeated freeze-thaw cycles.
c. Sodium Azide is incompatible with this assay.
d. Sample dilution guidelines: The user needs to estimate the concentration of β-catenin in the sample and select a proper dilution factor so that the diluted β-catenin concentration falls near the middle of the linear regime of the standard curve. Dilute the sample using the

provided sample diluent. The sample must be well mixed with the diluent buffer. Several trials may be necessary to optimize sample dilution. Suggested dilution: 1:5. Add 10 µl sample and 40 µl Sample diluent, mix gently without touching the walls of the plate.

VIII. Assay Protocol:

The 96-well plate should not be dry at any time, as drying will inactivate the active components on the plate.

1. Add 50 µl per well of the six human β-catenin standard solutions in the pre-coated 96-well plate. Add 50 µl sample diluent buffer into the sample control well (Zero well). Add 50 µl each of the 1:5 or properly diluted samples of human cell culture medium, cell or tissue lysate, serum or plasma to each empty well. See "Sample Dilution Guideline" for details.

   Notes:

   a. We recommend that each human β-catenin standard solution and each sample be measured in duplicate

   b. We recommend doing a pilot experiment using standards and a small number of samples to validate the assay procedure with the specific samples and optimize the appropriate sample dilution.

2. Cover the plate with the plate cover and incubate at 37°C for 30 min. Remove the cover, discard plate contents, and blot the plate onto paper towels or other absorbent material. Do not let the wells completely dry at any time.
3. Add 0.35 ml of prepared working wash solution into each well. Wash plate 5 times, each time leave washing buffer in the wells for 1-2 mins. Discard the washing buffer and blot the plate onto paper towels or other absorbent material. Always drain excess wash solution without drying the wells.
4. Add 50 µl of HRP-conjugate reagent into each well (except the Zero well) and incubate plate at 37°C in dark for 30 min.

   Note: These guidelines are for reference only; the optimal incubation time should be determined by end user. The shades of blue can be seen in the wells with the most concentrated human β-catenin standard solutions. The other wells might not show any obvious color.
5. Discard the HRP solution and wash the wells as described in Step 3.
6. Add 50 µl of Chromogen solution A and 50 µl of Chromogen solution B into each well. Incubate plate at 37°C in dark for 10 mins. or as required.
7. Add 50 µl of stop solution into each well. The color changes from blue to yellow immediately.
8. Read absorbance at 450 nm in a microplate reader within 15 min. after adding the stop solution.
9. Calculation: Relative $O.D._{450}$ = $O.D._{450}$ of each well – $O.D._{450}$ of Zero well. The standard curve can be plotted as the relative $O.D._{450}$ of each standard solution (Y) vs. the respective concentration of the standard solution (X). The human β-catenin concentration of the samples can be interpolated from the standard curve. **Note:** if the samples were diluted, multiply the dilution factor to the concentrations from interpolation to obtain the original concentration in the sample.

Typical Data Obtained from Human β-catenin
(Reaction incubated at 37°C for 30 min.)

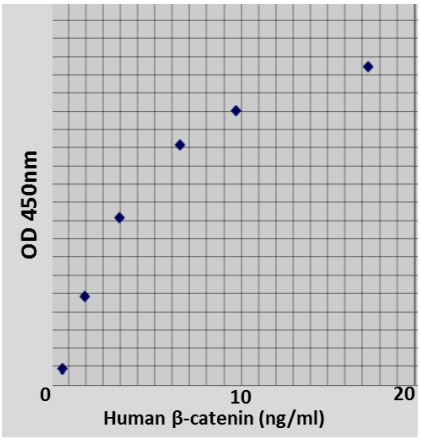

Figure: Standard Curve: This standard curve is for demonstration only. A standard curve must be run with each assay.

IX. RELATED PRODUCTS:

β-catenin (mouse) ELISA Kit (K3382-100)
Cadherin Antibody (3365R-100)
LRP-5/6 Antibody (3714)
LRP-5/6 Antibody , Clone 1A12 (3801)
Phospho-β-catenin antibody (3381-100)
β-catenin (rat) ELISA Kit (K3383-100)
CRT Inhibitor, iCRT5 (1896)
IWP-3 (2349)
Kenpaullone (1904)

FOR RESEARCH USE ONLY! Not to be used on humans.