

## ab285328 – Human $\beta$ -catenin ELISA Kit

For the quantitative detection of  $\beta$ -catenin in human serum, cell culture mediums and other biological fluids.

For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit:

<http://www.abcam.com/ab285328>

### Storage and Stability

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.

### Materials Supplied

Item	Quantity	Storage Condition
96 wells coated with anti-human $\beta$ -catenin antibody, 1 Microplate	12 Strips x 8 wells	4°C
Human $\beta$ -catenin standard (18 ng/ml)	500 $\mu$ l	4°C
HRP-conjugate reagent	6 mL	4°C
Standard Diluent	1.5 mL	4°C
Sample Diluent	6 mL	4°C
Chromogen Solution A	6 mL	4°C
Chromogen Solution B	6 mL	4°C
Stop Solution	6 mL	4°C
Wash Solution (30x stock)	20 mL	4°C
Sealed Bag	1 Unit	4°C
Plate cover	2 units	4°C

### Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully utilize this assay:

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

### Reagent Preparation

- Prepare reagents within 30 minutes before the experiment.
- Before using the kit, spin tubes and bring down all components to the bottom of tubes.

**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.

**Δ Note:** *If there is precipitation in the wash solution, gently warm to 37°C to dissolve.*

### Standard Preparation

1. Label 6 tubes with 18, 9, 4.5, 2.25, 1.125 and 0.5625 ng/ml of  $\beta$ -catenin Standard. Add 50  $\mu$ l Standard Diluent to tubes 2-6.
  - a. Tube 1: Aliquot 150  $\mu$ l of the provided Human  $\beta$ -catenin Standard (18 ng/ml)
  - b. Tube 2: Add 50  $\mu$ l from Tube 1 and mix to make 9 ng/ml.
  - c. Tube 3: Add 50  $\mu$ l from Tube 2 and mix to make 4.5 ng/ml.
  - d. Tube 4: Add 50  $\mu$ l from Tube 3 and mix to make 2.25 ng/ml.
  - e. Tube 5: Add 50  $\mu$ l from Tube 4 and mix to make 1.125 ng/ml.
  - f. Tube 6: Add 50  $\mu$ l from Tube 5 and mix to make 0.5625 ng/ml

### Sample Preparation

- For all samples, aliquot and freeze samples at -80°C.
- Avoid repeated freeze-thaw cycles.
- Sodium Azide is incompatible with this assay.

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.

**Δ Note – Sample Dilution Guidelines:** *The user needs to estimate the concentration of  $\beta$ -catenin in the sample and select a proper dilution factor so that the diluted  $\beta$ -catenin concentration falls near the middle of the linear regime of the standard curve. Dilute the sample using 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  $\mu$ l sample and 40  $\mu$ l Sample diluent, mix gently without touching the walls of the plate.*

### Assay Procedure

- It is recommended that each human  $\beta$ -catenin standard solution and each sample be measured in duplicate b.
  - It is recommended to conduct 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.
1. Add 50  $\mu$ l per well of the six human  $\beta$ -catenin standard solutions in the pre-coated 96-well plate. Add 50  $\mu$ l sample diluent buffer into the sample control well (Zero well). Add 50  $\mu$ l 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 Guidelines" for details
  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. Measure the optical density (OD) at 450 nm wavelength within 15 minutes after adding the stop solution.  
**Δ 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.

## Calculations

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.

**Δ Note:** *if the samples were diluted, multiply the dilution factor to the concentrations from interpolation to obtain the original concentration in the sample*

Download our ELISA guide for technical hints, results, calculation, and troubleshooting tips:

[www.abcam.com/protocols/the-complete-elisa-guide](http://www.abcam.com/protocols/the-complete-elisa-guide)

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