

## 27-Hydroxycholesterol (27-HC) ELISA Kit

Catalogue No.: abx257403



27-Hydroxycholesterol (27-HC) ELISA Kit is an ELISA Kit for the in vitro quantitative measurement of 27-Hydroxycholesterol (27-HC) concentrations in serum, plasma, tissue homogenates, cell lysates and other biological fluids.

<b>Target:</b>	27-Hydroxycholesterol (27-HC)
<b>Reactivity:</b>	General
<b>Tested Applications:</b>	ELISA
<b>Recommended dilutions:</b>	Optimal dilutions/concentrations should be determined by the end user.
<b>Storage:</b>	Shipped at 4 °C. Upon receipt, store the kit according to the storage instruction in the kit's manual.
<b>Validity:</b>	The validity for this kit is 6 months.
<b>Stability:</b>	The stability of the kit is determined by the rate of activity loss. The loss rate is less than 5% within the expiration date under appropriate storage conditions. To minimize performance fluctuations, operation procedures and lab conditions should be strictly controlled. It is also strongly suggested that the whole assay is performed by the same user throughout.
<b>Test Range:</b>	15.6 ng/ml - 1000 ng/ml
<b>Sensitivity:</b>	< 9.38 ng/ml
<b>Standard Form:</b>	Lyophilized
<b>Detection Method:</b>	Colorimetric
<b>Assay Type:</b>	Competitive
<b>Assay Data:</b>	Quantitative

**Sample Type:** Serum, plasma, tissue homogenates, cell lysates and other biological fluids.

**Kit Components:**

- Pre-coated 96-Well Microplate
- Standard
- Standard Diluent Buffer
- Wash Buffer
- Detection Reagent A
- Detection Reagent B
- Diluent A
- Diluent B
- TMB Substrate
- Stop Solution
- Plate Sealer

**Material Required But Not Provided:**

- 37°C incubator
- Multi and single channel pipettes and sterile pipette tips
- Squirt bottle or automated microplate washer
- 1.5 ml tubes
- Distilled water
- Absorbent filter papers
- 100 ml and 1 liter graduated cylinders
- Microplate reader (wavelength: 450 nm)
- ELISA Shaker

**Sample Collection/Preparation:**

- **Serum:** Samples should be collected into a serum separator tube. Coagulate the serum by leaving the tube undisturbed in a vertical position overnight at 4°C or at room temperature for up to 60 minutes. Centrifuge at approximately 1000 × g for 20 min. Analyze the serum immediately or aliquot and store at -20°C or -80°C.
- **Plasma:** Collect plasma using heparin or EDTA as an anticoagulant. Centrifuge for 15 minutes at 1000 × g within 30 minutes of collection. Assay immediately or aliquot and store at -20°C or -80°C. Avoid hemolysis and high cholesterol samples.
- **Tissue homogenates:** The preparation of tissue homogenates will vary depending upon tissue type – this is just an example. Rinse tissues with ice-cold PBS to remove the excess of blood. Weigh before homogenization. Finely mince tissues and homogenize with a tissue homogenizer on ice in PBS and sonicate the cell suspension. Centrifuge the homogenates at 5000 × g for 5 min and collect the supernatant. Assay immediately or aliquot and store at -20°C.

**Reagent Preparation:**

- 1) **Standard:** Prepare the standard with the recommended volume of Standard Diluent Buffer, to make the standard solution. Then use the Standard Diluent buffer to carry out serial dilutions of the standard solution, as instructed in the Protocol.
- 2) **Wash Buffer:** Dilute the concentrated Wash Buffer with distilled water, as instructed in the Protocol.
- 3) **Detection Reagent Preparation:** Calculate the total volume of working solution required. Dilute Detection Reagent A and Detection Reagent B with Diluent A and Diluent B, respectively, at 1:100.

## Assay Procedure:

- 1) Set standard, test samples and control wells.
- 2) Aliquot 50  $\mu$ l of diluted standard into the standard wells.
- 3) Aliquot 50  $\mu$ l of Standard Diluent buffer into the control (zero) well.
- 4) Aliquot 50  $\mu$ l of diluted samples into the sample wells.
- 5) Immediately aliquot 50  $\mu$ l of Detection Reagent A to each well. Incubate for 45 mins at 37 °C.
- 6) Wash 3 times.
- 7) Aliquot 100  $\mu$ l of Detection Reagent B to each well. Incubate for 30 mins at 37 °C.
- 8) Wash 5 times.
- 9) Aliquot 90  $\mu$ l of TMB Substrate to each well. Incubate for 10-20 mins at 37 °C.
- 10) Aliquot 50  $\mu$ l of Stop Solution.
- 11) Measure the OD at 450 nm.

## Note:

This product is for research use only.

The range and sensitivity is subject to change. Please contact us for the latest product information. For accurate results, sample concentrations must be diluted to mid-range of the kit. If you require a specific range, please contact us in advance or write your request in your order comments.

Please note that our ELISA and CLIA kits are optimised for detection of native samples, rather than recombinant proteins. We are unable to guarantee detection of recombinant proteins, as they may have different sequences or tertiary structures to the native protein.

For Reference Only