



User's Manual

Human Legumain ELISA Kit



DEIA-XYZ163



96T



This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

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PRODUCT INFORMATION

Intended Use

The Human Legumain ELISA kit is an in vitro enzyme-linked immunosorbent assay for the quantitative measurement of human Legumain in serum, plasma and cell culture supernatants.

Principles of Testing

This assay employs an antibody specific for human Legumain coated on a 96- well plate. Standards and samples are pipetted into the wells and Legumain present in a sample is bound to the wells by the immobilized antibody. The wells are washed and biotinylated anti-human Legumain antibody is added. After washing away unbound biotinylated antibody, HRP-conjugated streptavidin is pipetted to the wells. The wells are again washed, a TMB substrate solution is added to the wells and color develops in proportion to the amount of Legumain bound. The Stop Solution changes the color from blue to yellow, and the intensity of the color is measured at 450 nm.

Storage

The entire kit may be stored at -20°C for up to 1 year from the date of shipment. Avoid repeated freeze-thaw cycles. The kit may be stored at 4°C for up to 6 months. For extended storage, it is recommended to store at -80°C.

Assay Procedure

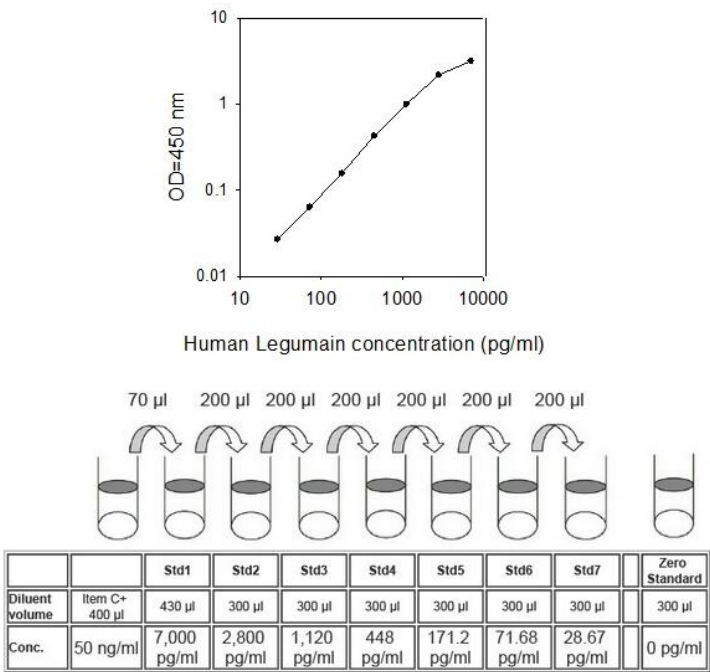
1. Prepare all reagents, samples and standards as instructed in the manual.
2. Add 100 µl of standard or sample to each well.
3. Incubate 2.5 h at RT or O/N at 4°C.
4. Add 100 µl of prepared biotin antibody to each well.
5. Incubate 1 h at RT.
6. Add 100 µl of prepared Streptavidin solution to each well.
7. Incubate 45 min at RT.
8. Add 100 µl of TMB One-Step Substrate Reagent to each well.
9. Incubate 30 min at RT.
10. Add 50 µl of Stop Solution to each well.
11. Read at 450 nm immediately.

Calculation

Calculate the mean absorbance for each set of duplicate standards, controls and samples, and subtract the average zero standard optical density. Plot the standard curve on log-log graph paper or using Sigma plot software, with standard concentration on the x-axis and absorbance on the y-axis. Draw the best-fit straight

line through the standard points.

Typical Standard Curve



Precision

Intra-Assay CV%: <10%
Inter-Assay CV%: <12%

Detection Range

28.7 pg/ml - 7,000 pg/ml

Sensitivity

28.7 pg/ml

Specificity

Cross Reactivity: This ELISA kit shows no cross-reactivity with the following cytokines tested: human 2B4 (CD244), ADAM-9, Angiopoietin-2, APRIL, BMP-2, BMP-9, C5a, Cathepsin L, CD200, CD97, Chemerin, DcR3, FABP2, FAP, FGF-19, Galectin-3, HGFR, IFN-alpha / beta R2, IGF-2, IGF-2 R, IL-1 R6 (IL-1 Rrp2), IL-24, IL-33 (IL-1 F11), Kallikrein 14.

Linearity

Sample Type		Serum	Plasma	Cell Culture Media
1:2	Average % of Expected Range (%)	73.95 70-78	71.19 68-75	104.6 95-114
1:4	Average % of Expected Range (%)	77.71 73-82	83.78 72-104	126.5 104-129

Recovery

Sample Type	Average % Recovery	Range (%)
Serum	103.4	69-129
Plasma	109.1	82-144
Cell culture media	124.4	118-133