



User's Manual

Human HAI-2 ELISA Kit



DEIA-XYZ134



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.

Creative Diagnostics

 Address: 45-1 Ramsey Road, Shirley, NY 11967, USA

 Tel: 1-631-624-4882 (USA) 44-161-818-6441 (Europe)  Fax: 1-631-938-8221

 Email: info@creative-diagnostics.com  Web: www.creative-diagnostics.com

PRODUCT INFORMATION

Intended Use

The Human HAI-2 ELISA kit is an in-vitro enzyme-linked immunosorbent assay for the quantitative measurement of human HAI-2 in serum, plasma (collect plasma using EDTA and Citrate as an anticoagulant; heparin is not recommended as an anticoagulant for use in this assay), and cell culture supernatants.

Principles of Testing

This assay employs an antibody specific for human HAI-2 coated on a 96-well plate. Standards and samples are pipetted into the wells and HAI-2 present in a sample is bound to the wells by the immobilized antibody. The wells are washed and biotinylated anti-human HAI-2 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 HAI-2 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

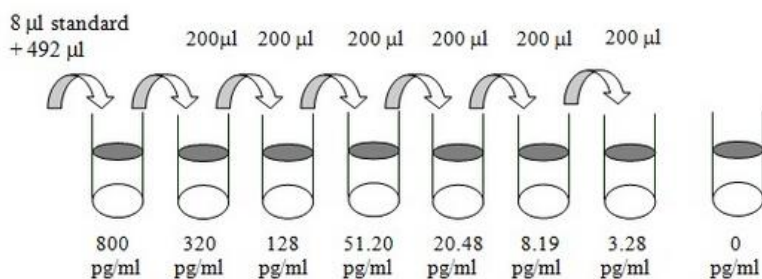
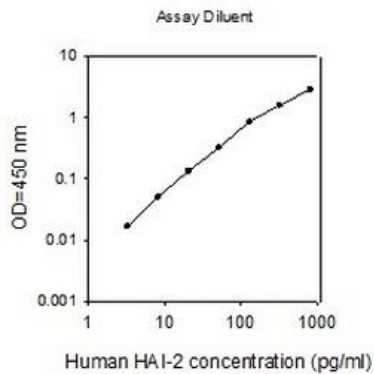
1. Prepare all reagents, samples and standards as instructed in the manual.
2. Add 100 µl of standard or sample to each well.
3. Incubate 2.5 h at RT or O/N at 4°C.
4. Add 100 µl of prepared biotin antibody to each well.
5. Incubate 1 h at RT.
6. Add 100 µl of prepared Streptavidin solution to each well.
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

3 pg/ml - 800 pg/ml

Sensitivity

3 pg/ml

Specificity

This ELISA antibody pair detects human HAI-2, less than 0.1% cross-reactivity with human HAI-1 and mouse HAI-2B is observed

Linearity

Sample Type		Serum	Plasma	Cell Culture Media
1:2	Average % of Expected Range (%)	119.9 113-127	114.2 109-119	118.8 104-126
1:4	Average % of Expected Range (%)	126.6 119-135	125.0 115-135	124.2 118-130

Recovery

Sample Type	Average % Recovery	Range (%)
Serum	73.36	68-84
Plasma	70.45	67-79
Cell culture media	78.51	69-91