Datasheet for ABIN6960627

IL12 Ab ELISA Kit

1 Image

Go to Product page

Overview

Quantity:	96 tests
Target:	IL12 Ab
Reactivity:	Human
Method Type:	Competition ELISA
Detection Range:	31.2 pg/mL - 2000 pg/mL
Minimum Detection Limit:	31.2 pg/mL
Application:	ELISA

Product Details

Purpose:	The kit is an enzyme immunoassay for in vitro quantitative measurement of Anti-IL12 in human serum, plasma.
Sample Type:	Plasma, Serum
Analytical Method:	Quantitative
Detection Method:	Colorimetric
Specificity:	This assay has high sensitivity and excellent specificity for detection of Anti-Interleukin 12 Antibody (Anti-IL12)
Sensitivity:	11.6 pg/mL
Components:	• Pre-coated, ready to use 96-well strip plate, flat buttom • Plate sealer for 96 wells • Reference Standard • Standard Diluent

Product Details

- Detection Reagent A
- Detection Reagent B
- Assay Diluent A
- Assay Diluent B
- Reagent Diluent (if Detection Reagent is lyophilized)
- TMB Substrate
- Stop Solution
- Wash Buffer (30 x concentrate)
- Instruction manual

Target Details

Target:	IL12 Ab
Alternative Name:	Anti-Interleukin 12 Antibody (Anti-IL12) (IL12 Ab Products)
Target Type:	Antibody

Application Details

Comment:	Information on standard material: The standard might be recombinant protein or natural protein, that will depend on the specific kit. Moreover, the expression system is E.coli or yeast or mammal cell. There is 0.05% proclin 300 in the standard as preservative. Information on reagents: The stop solution used in the kit is sulfuric acid with concentration of 1 mol/L. And the wash solution is TBS. The standard diluent contains 0.02 % sodium azide, assay diluent A and assay diluent B contain 0.01% sodium azide. Some kits can contain is BSA in them. Information on antibodies: The provided antibodies and their host vary in different kits.
Sample Volume:	50 µL
Assay Time:	2 h
Plate:	Pre-coated
Protocol:	1. Prepare all reagents, samples and standards, 2. Add 50µL standard or sample to each well. And then add 50µL prepared Detection Reagent A immediately.

Shake and mix. Incubate 1 hour at 37 °C,
3. Aspirate and wash 3 times,
4. Add 100µL prepared Detection Reagent B. Incubate 30 minutes at 37 °C,
5. Aspirate and wash 5 times,
6. Add 90µL Substrate Solution. Incubate 10-20 minutes at 37 °C,
7. Add 50µL Stop Solution. Read at 450 nm immediately.

Reagent Preparation:

1. Bring all kit components and samples to room temperature (18-25 °C) before use.
2. Standard - Reconstitute the Standard with 0.4 mL of Standard Diluent, kept for 10 minutes at room temperature, shake gently(not to foam). The concentration of the standard in the stock solution is 2,000pg/mL. Please prepare 7 tubes containing 0.25 mL Standard Diluent and produce a double dilution series according to the picture shown below. Mix each tube thoroughly before the next transfer. Set up 7 points of diluted standard such as 2,000pg/mL, 1,000pg/mL, 500pg/mL, 250pg/mL, 125pg/mL, 62.5pg/mL, 31.2pg/mL, and the last EP tubes with Standard Diluent is the blank as 0pg/mL.
3. Detection Reagent A - Briefly spin or centrifuge the stock Detection A before use. Dilute to the working concentration with working Assay Diluent A, respectively (1:100).
4. Wash Solution - Dilute 20 mL of Wash Solution concentrate (30x) with 580 mL of deionized or distilled water to prepare 600 mL of Wash Solution (1x).
5. TMB substrate - Aspirate the needed dosage of the solution with sterilized tips and do not dump the residual solution into the vial again.

Note:

1. The standard for this kit is liquid. Due to its small volume, maybe invisible to the eye.
2. Making serial dilution in the wells directly is not permitted.
3. Prepare standard within 15 minutes before assay. Please do not dissolve the reagents at 37 °C directly.
4. Please carefully reconstitute Standards or working Detection Reagent A according to the instruction, and avoid foaming and mix gently until the crystals are completely dissolved. To minimize imprecision caused by pipetting, use small volumes and ensure that pipettors are calibrated. It is recommended to suck more than 10µL for once pipetting.
5. The reconstituted Standards and Detection Reagent A can be used only once.
6. If crystals have formed in the Wash Solution concentrate (30x), warm to room temperature and mix gently until the crystals are completely dissolved.
7. Contaminated water or container for reagent preparation will influence the detection result.

Sample Preparation:

- It is recommended to use fresh samples without long storage, otherwise protein degradation and denaturationmay occur in these samples, leading to false results. Samples should therefore be stored for a short periodat 2 - 8 °C or aliquoted at -20 °C (≤1 month) or -80 °C (≤ 3 months). Repeated freeze-thawcycles should be avoided. Prior to assay, the frozen samples should be slowly thawed and centrifuged toremove precipitates.
- If the sample type is not specified in the instructions, a preliminary test is necessary to determinecompatibility with the kit.
- If a lysis buffer is used to prepare tissue homogenates or cell culture supernatant, there is a

possibilityof causing a deviation due to the introduced chemical substance.The recommended dilution factor is for reference only.

- Please estimate the concentration of the samples before performing the test. If the values are not in therange of the standard curve, the optimal sample dilution for the particular experiment has to be determined.Samples should then be diluted with PBS (pH =7.0-7.2).

Assay Procedure:

1. Determine wells for diluted standard, blank and sample. Prepare wells for standard points and blank. Add 50µL each of dilutions of standard (read Reagent Preparation), blank and samples into the appropriate wells, respectively. And then add 50µL of Detection Reagent A to each well immediately. Shake the plate gently (using a microplate shaker is recommended). Cover with a Plate sealer. Incubate for 1 hour at 37 °C. Detection Reagent A may appear cloudy. Warm to room temperature and mix gently until solution appears uniform.
2. Aspirate the solution and wash with 350µL of 1X Wash Solution to each well using a squirt bottle, multi-channel pipette, manifold dispenser or autowasher, and let it sit for 1-2 minutes. Remove the remaining liquid from all wells completely by snapping the plate onto absorbent paper. Repeat 3 times. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against absorbent paper.
3. Add 100µL of Detection Reagent B working solution to each well. Incubate for 30 minutes at 37 °C after covering it with the Plate sealer.
4. Repeat the aspiration/wash process for total 5 times as conducted in step 2.
5. Add 90µL of Substrate Solution to each well. Cover with a new Plate sealer. Incubate for 15 - 25 minutes at 37 °C (Don't exceed 30 minutes). Protect from light. The liquid will turn blue by the addition of Substrate Solution.
6. Add 50µL of Stop Solution to each well. The liquid will turn yellow by the addition of Stop solution. Mix the liquid by tapping the side of the plate. If color change does not appear uniform, gently tap the plate to ensure thorough mixing.
7. Remove any drop of water and fingerprint on the bottom of the plate and confirm there is no bubble on the surface of the liquid. Then, run the microplate reader and conduct measurement at 450nm immediately.

Note:

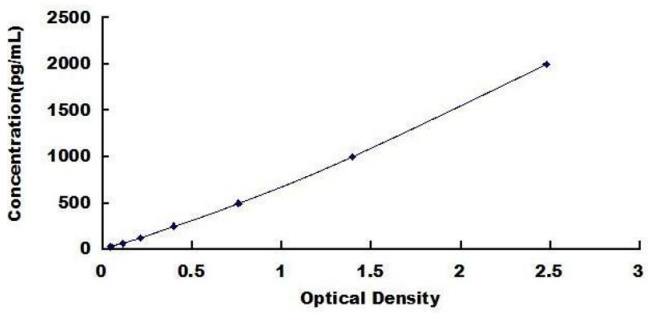
1. Assay preparation: Keep appropriate numbers of wells for each experiment and remove extra wells from microplate. Rest wells should be resealed and stored at -20 °C.
2. Samples or reagents addition:Please use the freshly prepared Standard. Please carefully add samples to wells and mix gently to avoid foaming. Do not touch the well wall. For each step in the procedure, total dispensing time for addition of reagents or samples to the assay plate should not exceed 10 minutes. This will ensure equal elapsed time for each pipetting step, without interruption. Duplication of all standards and specimens, although not required, is recommended. To avoid cross-contamination, change pipette tips between additions of standards, samples, and reagents. Also, use separated reservoirs for each reagent.
3. Incubation: To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary. Do not allow wells to sit uncovered for extended periods between incubation steps. Once reagents are added to the well strips, DO NOT let the strips DRY at

	any time during the assay. Incubation time and temperature must be controlled. 4. Washing: The wash procedure is critical. Complete removal of liquid at each step is essential for good performance. After the last wash, remove any remaining Wash Solution by aspirating or decanting and remove any drop of water and fingerprint on the bottom of the plate. Insufficient washing will result in poor precision and false elevated absorbance reading. 5. Controlling of reaction time: Observe the change of color after adding TMB Substrate (e.g. observation once every 10 minutes), if the color is too deep, add Stop Solution in advance to avoid excessively strong reaction which will result in inaccurate absorbance reading. 6. TMB Substrate is easily contaminated. Please protect it from light. 7. The environment humidity which is less than 60 % might have some effects on the final performance, therefore, a humidifier is recommended to be used at that condition.
Calculation of Results:	This assay employs the competitive inhibition enzyme immunoassay technique, so there is an inverse correlation between target concentration in the sample and the assay signal intensity. Average the duplicate readings for each standard, control, and samples. Create a standard curve on log-log or semi-log graph paper, with the log of target concentration on the y-axis and absorbance on the x-axis. Draw the best fit straight line through the standard points and it can be determined by regression analysis. Using some plot software, for instance, curve expert 1.30, is also recommended. If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor. In order to make the calculation easier, we plot the O.D. value of the standard (X-axis) against the log of concentration of the standard (Y-axis), although concentration is the independent variable and O.D. value is the dependent variable. The O.D. values of the standard curve may vary according to the conditions of assay performance (e.g. operator, pipetting technique, washing technique or temperature effects). Typical standard curve below is provided for reference only.
Assay Precision:	Intra-assay Precision (Precision within an assay): 3 samples with low, middle and high level of target were tested 20 times on one plate, respectively. Inter-assay Precision (Precision between assays): 3 samples with low, middle and high level of target were tested on 3 different plates, 8 replicates in each plate. CV(%) = SD/meanX100 Intra-Assay: CV < 10% Inter-Assay: CV < 12%
Restrictions:	For Research Use only

Handling

Precaution of Use:	The Stop Solution suggested for use with this kit is an acid solution. Wear eye, hand, face, and clothing protection when using this material.
Storage:	4 °C/-20 °C
Storage Comment:	1. For unopened kit: All reagents should be stored according to the labels on the vials. The Standard, Detection Reagent A, Detection Reagent B, and 96-well Strip Plate should be stored at -20 °C upon receipt, while the other reagents should be stored at 4 °C. 2. For opened kits: the remaining reagents must be stored according to the above storage conditions. In addition, please return the unused wells to the foil pouch containing the desiccant and seal the foil pouch with the zipper. .
Expiry Date:	6 months

Images



ELISA

Image 1. Typical standard curve