
Human CD3 ELISA kit

Catalog No.E1872h

(96 tests)

Operating instruction

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PLEASE READ THROUGH ENTIRE PROCEDURE BEFORE BEGINNING!**

Intended use

This immunoassay kit allows for the in vitro quantitative determination of human CD3 concentrations in cell culture supernates, and other biological fluids.

Introduction

In immunology, the CD3 antigen (CD stands for cluster of differentiation) is a protein complex composed of four distinct chains (CD3 γ , CD3 δ and two times CD3 ϵ) in mammals, that associate with molecules known as the T cell receptor (TCR) and the ζ -chain to generate an activation signal in T lymphocytes. The TCR, ζ -chain and CD3 molecules together comprise the TCR complex. The CD3 γ , CD3 δ and CD3 ϵ chains are highly related cell surface proteins of the immunoglobulin superfamily containing a single extracellular immunoglobulin domain. The transmembrane region of the CD3 chains is negatively charged, a characteristic that allows these chains to associate with the positively charged TCR chains (TCR α and TCR β). The intracellular tails of the CD3 molecules contain a single conserved motif known as an immunoreceptor tyrosine-based activation motif or ITAM for short, which is essential for the signaling capacity of the TCR. Phosphorylation of the ITAM on CD3 renders the CD3 chain capable of binding an enzyme called ZAP70 (zeta associated protein), a kinase that is important in the signaling cascade of the T cell.

Test principle

The microtiter plate provided in this kit has been pre-coated with an antibody specific to CD3. Standards or samples are then added to the appropriate microtiter plate wells with a biotin-conjugated polyclonal antibody preparation specific for CD3 and Avidin conjugated to Horseradish Peroxidase (HRP) is added to each microplate well and incubated. Then a TMB (3,3',5,5' tetramethyl-benzidine) substrate solution is added to each well. Only those wells that contain CD3, biotin-conjugated antibody and enzyme-conjugated Avidin will exhibit a change in color. The enzyme-substrate reaction is terminated by the addition of a sulphuric acid solution and the color change is measured spectrophotometrically at a wavelength of 450 nm \pm 2 nm. The concentration of CD3 in the samples is then determined by comparing the O.D. of the samples to the standard curve.

Materials and components

Reagent	Quantity
Assay plate	1

Standard	2
Sample Diluent	1 x 20ml
Assay Diluent A	1 x 10ml
Assay Diluent B	1 x 10ml
Detection Reagent A	1 x 120ul
Detection Reagent B	1 x 120ul
Wash Buffer	1 x 30ml
(25 x concentrate)	
Substrate	1 x 10ml
Stop Solution	1 x 10ml

Sample collection and storage

Cell culture supernates and other biological fluids - Remove particulates by centrifugation and assay immediately or aliquot and store samples at -20° C or -80° C. Avoid repeated freeze-thaw cycles.

Note: cell culture supernatant samples to be used within 7 days may be stored at 2-8 ° C, otherwise samples must be stored at -20° C (\leq 1 months) or -80° C (\leq 2 months) to avoid loss of bioactivity and contamination. Avoid freeze-thaw cycles. When performing the assay slowly bring samples to room temperature.

It is recommended that all samples be assayed in duplicate.

DO NOT USE HEAT-TREATED SPECIMENS.

Limitations of the procedure

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1. The kit should not be used beyond the expiration date on the kit label.
2. Do not mix or substitute reagents with those from other lots or sources.
3. If samples generate values higher than the highest standard, further dilute the samples with the Assay Diluent and repeat the assay. Any variation in standard diluent, operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.
4. This assay is designed to eliminate interference by soluble receptors, ligands, binding proteins, and other factors present in biological samples. Until all factors have been tested in the Quantikine Immunoassay, the possibility of interference cannot be excluded.

Reagent preparation

Bring all reagents to room temperature before use.

Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Dilute 30 mL of Wash Buffer Concentrate into deionized or distilled water to prepare 750 mL of Wash Buffer.

Standard - Reconstitute the **Standard** with 1.0 mL of **Sample Diluent**. This reconstitution produces a stock solution of 100 ng/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making serial dilutions. The undiluted standard serves as the high standard (100 ng/mL). The **Sample Diluent** serves as the zero standard (0 ng/mL).

Detection Reagent A and B - Dilute to the working concentration using **Assay Diluent A and B**

(1:100), respectively.

Assay procedure

Allow all reagents to reach room temperature. **All the reagents should be mixed thoroughly by gently swirling before pipetting. Avoid foaming.** Arrange and label required number of strips. Prepare all reagents, working standards and samples as directed in the previous sections.

1. Add 100 uL of **Standard**, Blank, or Sample per well. Cover with the adhesive strip. Incubate for 2 hours at 37° C.
2. Remove the liquid of each well, don't wash.
3. Add 100 uL of **Detection Reagent A** working solution to each well. Incubate for 1 hour at 37°C. **Detection Reagent A** working solution may appear cloudy. Warm to room temperature and mix gently until solution appears uniform.
4. Aspirate each well and wash, repeating the process three times for a total of three washes. Wash by filling each well with Wash Buffer (350 uL) using a squirt bottle, multi-channel pipette, manifold dispenser or autowasher. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
5. Add 100 uL of **Detection Reagent B** working solution to each well. Cover with a new adhesive strip. Incubate for 1 hours at 37° C.
6. Repeat the aspiration/wash as in step 4.
7. Add 90 uL of **Substrate Solution** to each well. Incubate within 30 minutes at 37°C. Protect from light.
8. Add 50 uL of **Stop Solution** to each well. If color change does not appear uniform, gently tap the plate to ensure thorough mixing.
9. Determine the optical density of each well at once, using a microplate reader set to 450 nm.

Specificity

This assay recognizes recombinant and natural human CD3. No significant cross-reactivity or interference was observed.

Sensitivity

The minimum detectable dose of human CD3 is typically less than 1 ng/mL.

The sensitivity of this assay, or Lower Limit of Detection (LLD) was defined as the lowest protein concentration that could be differentiated from zero.

Detection Range

1.56 -100 ng/mL. The standard curve concentrations used for the ELISA's were 100 ng/mL, 50 ng/mL, 25 ng/mL, 12.5 ng/mL, 6.25 ng/mL, 3.12 ng/mL, 1.56 ng/mL.

Important Note:

1. Please carefully reconstitute **Standards** or working **Detection Reagent A and B** according to the instruction, and avoid foaming and mix gently until the crystals have completely dissolved. The reconstituted **Standards** can be used only once.
2. The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.

3. It is recommended that no more than 32 wells be used for each assay run if manual pipetting is used since pipetting of all standards, specimens and controls should be completed within 5 minutes. A full plate of 96 wells may be used if automated pipetting is available.
4. Duplication of all standards and specimens, although not required, is recommended.
5. When mixing or reconstituting protein solutions, always avoid foaming.
6. To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.
7. To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
8. Do not substitute reagents from one kit lot to another. Use only the reagents supplied by manufacturer.

Calculation of results

Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density. Create a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the log of the CD3 concentrations versus the log of the O.D. and the best fit line can be determined by regression analysis. This procedure will produce an adequate but less precise fit of the data. If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

Storage of test kits and instrumentation

1. Unopened test kits should be stored at 2-8°C upon receipt and the microtiter plate should be kept in a sealed bag with desiccants to minimize exposure to damp air. The test kit may be used throughout the expiration date of the kit (six months from the date of manufacture). Refer to the package label for the expiration date.
2. Opened test kits will remain stable until the expiring date shown, provided it is stored as prescribed above.
3. Do not remove microtiter plate from the storage bag until needed. Unused strips should be stored at 2-8°C in their pouch with the desiccant provided.
4. A microtiter plate reader with a bandwidth of 10nm or less and an optical density range of 0-3 OD or greater at 450nm wavelength is acceptable for use in absorbance measurement.
5. Use fresh disposable pipette tips for each transfer to avoid contamination.
6. Substrate Solution is easily contaminated. If bluish prior to use, do not use.

Precaution

The Stop Solution suggested for use with this kit is an acid solution. Wear eye, hand, face, and clothing protection when using this material.