Product Manual

Human TGF-α (Transforming Growth Factor α) ELISA Kit

Catalog #: ENZ-KIT132

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Product Manual

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Please read entire booklet before proceeding with the assay.

Carefully note the handling and storage conditions of each kit component.

Please contact Enzo Life Sciences Technical Support if necessary and provide lot # from outside of kit box.

INTENDED USE

This ELISA kit applies to the in vitro quantitative determination of Human TGF-α concentrations in serum, plasma and other biological fluids.

SENSITIVITY

The minimum detectable dose of Human TGF-α is 9.375pg/mL (The sensitivity of this assay, or lowest detectable limit (LDL) was defined as the lowest protein concentration that could be differentiated from zero).

DETECTION RANGE

15.625-1000 pg/ml

SPECIFICITY

This kit recognizes natural and recombinant Human TGF-α. No significant cross-reactivity or interference between Human TGF-α and analogues were observed.

NOTE

Limited by existing techniques, cross-reaction may still exist, as it is impossible for us to complete the cross-reactivity detection between Human TGF-α and all the analogues.

REPRODUCIBILITY

Coefficient of variation was <10%.

STATEMENT

Thank you for choosing our products. This product is produced by using raw material from world-renowned manufacturer and professional manufacturing technology of ELISA kits. Please read the instructions carefully before use and check all the reagent compositions. If there are any concerns please contact Enzo Life Sciences.

Reagents require separate storage conditions.

Protect from light

STORAGE

All the reagents in the kit should be stored according to the labels on vials. Unused wells should be returned to the foil pouch with the desiccant pack and resealed along entire edge of zip-seal. The substrate reagent should not be kept at -20°C. Exposure of reagents to strong light should be avoided in the process of incubation and storage. All the reagent caps should be tightened to prevent evaporation and microbial contamination. Improper storage may result in loss of kit functionality.

MATERIALS SUPPLIED

Item	Specifications	Storage
Microplate	8 wells ×12 strips	4°C
Reference Standard	2 vials	4°C
Reference Standard & Sample Diluent	20ml	4°C
Concentrated Biotinylated Detection Ab	120µl	4°C
Biotinylated Detection Ab Diluent	10ml	4°C
Concentrated HRP Conjugate	120µl	4°C (protect from light)
HRP Conjugate Diluent	10ml	4°C
Concentrated Wash Buffer (25×)	30ml	4°C
Substrate Reagent	10ml	4°C (protect from light)
Stop Solution	10ml	4°C
Plate Sealer	5 pieces	
Manual	1 copy	

TEST PRINCIPLE

This ELISA kit uses a sandwich format. The microplate provided in this kit has been pre-coated with an antibody specific to TGF-α. Standards or samples are added to the appropriate microplate wells and combined with the specific antibody. Then a biotinylated detection antibody specific for TGF-α and Avidin-Horseradish Peroxidase (HRP) conjugate is added to each microplate well successively and incubated. Free components are washed away. The substrate solution is added to each well. Only those wells that contain TGF-α, biotinylated detection antibody and Avidin-HRP conjugate will appear blue in color. The enzyme-substrate reaction is terminated by the addition of a sulphuric acid solution and the color turns yellow. The optical density (OD) is measured spectrophotometrically at a wavelength of 450nm ± 2nm. The OD value is proportional to the concentration of TGF-α. You can calculate the concentration of TGF-α in the samples by comparing the OD of the samples to the standard curve.

SAMPLE COLLECTION AND STORAGE

Samples should be clear and transparent and be centrifuged to remove suspended solids.

Serum: Allow samples to clot for 2 hours at room temperature or overnight at 4°C before centrifugation for 15 minutes at 1000×g. Collect the supernatant and carry out the assay immediately. Blood collection tubes should be disposable, non-pyrogenic, and non-endotoxin.

Plasma: Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge samples for 15 minutes at 1000×g at 2–8°C within 30 minutes of collection. Collect the supernatant and carry out the assay immediately. Hemolysis samples are not suitable for ELISA assay!

Cell culture supernatant: Centrifuge supernatant for 20 minutes to remove insoluble impurity and cell debris at 1000×g at 2–8°C. Collect the clear supernatant and carry out the assay immediately.

Tissue homogenates: It is recommended to obtain additional details from literature references for different tissue types. When using different tissue types, you might want to obtain additional details from literature reference. For general information, hemolysis blood may affect the result, so you should mince the tissues to small pieces and rinse them in ice-cold PBS (0.01M, pH=7.4) to remove excess blood thoroughly. Tissue pieces should be weighed and then homogenized in PBS (the volume depends on the weight of the tissue) with a glass homogenizer on ice. To further break the cells, you can sonicate the suspension with an

ultrasonic cell disrupter or subject it to freeze-thaw cycles. The homogenates are then centrifuged for 5 minutes at 5000×g to get the supernatant.

Other biological fluids: Centrifuge samples for 20 minutes at 1000×g at 2–8°C. Collect the supernatant and carry out the assay immediately.

Note:

1. Samples should be used within 7 days when stored at 2-8°C, otherwise samples must be divided and stored at -20°C (≤ 1month) or -80°C (≤ 6months) to avoid the loss of bioactivity and contamination. Avoid repeated freeze-thaw cycles.
2. Bring the samples to room temperature (18-25°C) without extra heating before performing the assay.
3. Predict the concentration before assaying. If the sample concentration is not within the range of the standard curve, users must determine the optimal sample dilutions for their particular experiments.

SAMPLE PREPARATION

1. Enzo Life Sciences is only responsible for the kit itself, and not for the samples consumed during the experiment. The user should calculate the amount of samples needed. Reserving sufficient samples in advance is recommended.
2. If the samples are not mentioned in this manual, a pre-experiment to determine the validity of the kit is necessary.
3. Tissue or cell extraction samples prepared by chemical lysis buffer may cause unexpected ELISA results due to the impact of certain chemicals.
4. Due to the possibility of mismatching between antigen from other origins and antibodies used in our kits, some native or recombinant proteins from other manufacturers may not be detected by our kits.
5. For cell culture supernatants, factors including cell viability, cell number or sampling time will affect results.
6. Grossly hemolyzed samples are not suitable for use in the assay.
7. Fresh samples are recommended for use with this kit.

OTHER MATERIALS NEEDED

- Microplate reader with 450nm wavelength filter
- High-precision transfer pipette, EP tubes and disposable pipette tips
- 37°C Incubator
- Deionized or distilled water
- Absorbent paper
- Loading slot for Wash Buffer

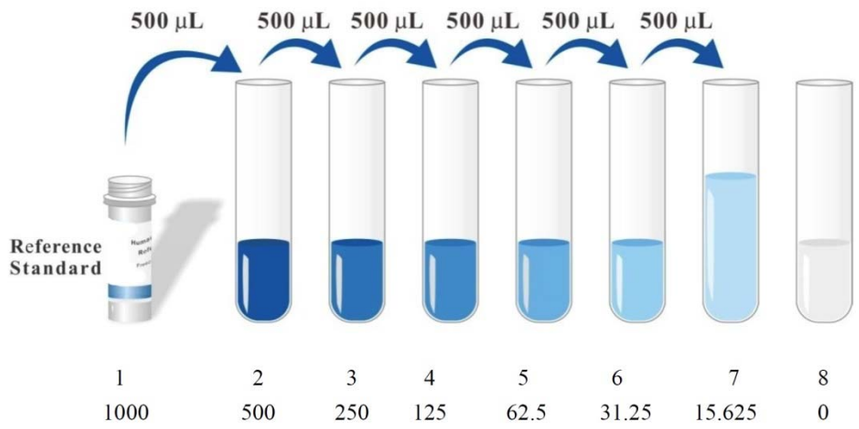
REAGENT PREPARATION

Bring all reagents to room temperature (18-25°C) before use.

Wash Buffer: Dilute 30 ml of Concentrated Wash Buffer into 750 ml of Wash Buffer with deionized or distilled water. Put unused solution back at 4°C. If crystals have formed in the concentrate, you can warm it in a 40°C water bath (Heating temperature should not exceed 50°C) and mix gently until the crystals have completely dissolved. The solution should be cooled to room temperature before use.

Standard: Prepare standard within 15 minutes before use. Centrifuge at 10,000×g for 1 minute, and reconstitute the Standard with 1.0 ml of Reference Standard & Sample Diluent. Tighten the lid, let it stand for 10 minutes and turn it upside down several times. After it dissolves fully, mix it thoroughly with a pipette. This reconstitution produces a stock solution of 1000 pg/ml. Then make serial dilutions as needed (making serial dilution in the wells directly is not recommended). The recommended concentrations are as follows: **100, 50, 25, 12.5, 6.25, 3.125, 1.563, 0 pg/ml**. If you want to make standard solution at the concentration of 50 pg/ml, you should take 0.5 ml standard at 100pg/ml, add it to an EP tube with 0.5 ml Reference Standard & Sample Diluent, and mix it. Procedures to prepare the remaining concentrations are all the same. The undiluted standard serves as the highest standard (100 pg/ml). The Reference Standard & Sample Diluent serves as the zero (0 pg/ml).

(Standards can also be diluted according to the actual amount, such as 200 µl/tube)



Biotinylated Detection Ab: Calculate the required amount before experiment (100 µl/well). In actual preparation, you should prepare 100~200 µl more. Centrifuge the stock tube before use; dilute the concentrated Biotinylated Detection Ab to the working concentration using Biotinylated Detection Ab Diluent (1:100).

Concentrated HRP Conjugate: Calculate the required amount before experiment (100 µl/well). In actual preparation, you should prepare 100~200µl more. Dilute the Concentrated HRP Conjugate to the working concentration using Concentrated HRP Conjugate Diluent (1:100).

Substrate Reagent: As it is sensitive to light and contaminants, do not open the vial until needed. The needed dosage of the reagent can be aspirated with sterilized tips and the unused residual reagent should not be placed back into the vial again.

Note: Please do not prepare the reagent directly in the Diluent vials provided in the kit. Contaminated water or container for reagent preparation will influence the result.

Washing Procedure:

1. **Automated Washer**: Add 350µl wash buffer into each well, the interval between injection and suction should be set about 60.
2. **Manual wash**: Add 350 µl Wash Buffer into each well, soak it for 1~2minutes. After the last wash, decant any remaining Wash Buffer by inverting the plate and blotting it dry by tapping it firmly against clean absorbent paper on a hard surface.

ASSAY PROCEDURE

Bring all reagents and samples to room temperature before use. Centrifuge the sample again after thawing, right before performing the assay. **Before pipetting, all the reagents should be mixed thoroughly by gently swirling. Avoid foaming.** It's recommended that all samples and standards be assayed in duplicate.

1. **Add Sample**: Add 100µl of Standard, Blank, or Sample per well. Reference Standard & Sample diluent is added to the blank well. Solutions are added to the bottom of microplate well, avoid touching the inside wall and foaming as much as possible. Mix it gently. Cover the plate with sealer provided. Incubate for 90 minutes at 37°C.
2. **Biotinylated Detection Ab**: Remove the liquid from each well, do not wash. Immediately add 100 µl of Biotinylated Detection Ab working solution to each well. Cover with the plate sealer. Gently tap the plate to ensure thorough mixing. Incubate for 1 hour at 37°C.
3. **Wash**: Aspirate each well and wash, repeating the process three times. Wash by filling each well with Wash Buffer (approximately 350 µl) (a squirt bottle, multi-channel pipette, manifold dispenser or automated washer are needed). Complete removal of liquid at each step is essential. After the last wash, remove remaining Wash Buffer by aspirating or decanting. Invert the plate and pat it against thick clean absorbent paper.
4. **HRP Conjugate**: Add 100 µl of HRP Conjugate working solution to each well. Cover with the Plate sealer. Incubate for 30 minutes at 37°C.
5. **Wash**: Repeat the wash process for five times as conducted in step 3.
6. **Substrate**: Add 90µl of Substrate Solution to each well. Cover with a new plate sealer. Incubate for about 15 minutes at 37°C. Protect the plate from light. The reaction time can be shortened or extended according to the actual color change, but not more than 30 minutes. When an apparent gradient appears in standard wells, user should terminate the reaction.
7. **Stop**: Add 50 µl of Stop Solution to each well. The color should turn to yellow immediately. The order to add the stop solution should be the same as the substrate solution.

8. **OD Measurement**: Determine the optical density (OD value) of each well at once, using a microplate reader set to 450nm. User should open the microplate reader in advance, preheat the instrument, and set the testing parameters.
9. After the experiment, put all the unused reagents back into the refrigerator according to the specified storage temperature respectively until their expiry.

IMPORTANT NOTES

1. **ELISA Plate**: The freshly opened ELISA plate may appear to contain a water-like substance, which is normal and will not have any impact on the experimental results.
2. **Add Sample**: The interval of sample adding between the first well and the last well should not be too long, otherwise this will cause different pre-incubation time, which will significantly affect the experiment's accuracy and reproducibility. For each step in the procedure, total dispensing time for addition of reagents or samples to the assay plate should not exceed 10 minutes. Parallel measurement is recommended.
3. **Incubation**: To prevent evaporation and 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. Do not let the strips dry at any time during the assay. Strict compliance with the given incubation time and temperature is important
4. **Washing**: The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings. Residual liquid in the reaction wells should be patted dry against absorbent paper in the washing process. But don't put absorbent paper into reaction wells directly. Note to clear any residual liquid and fingerprint from the bottom of the plate before measurement, as not to affect the micro-titer plate reader.
5. **Reagent Preparation**: As the volume of Concentrated Biotinylated Detection Ab and Concentrated HRP Conjugate is very small, liquid may adhere to the tube wall or tube cap when being transported. Spin for 1 minute at 1000 rpm. Pipette the solution for 4-5 times up and down before pipetting. Carefully reconstitute Standards, working solutions of Detection Ab and HRP Conjugate according to the instructions. To minimize imprecision caused by pipetting, ensure that pipettes are calibrated. It is recommended to pipette more than 10µl at a time. Do not

Avoid freeze / thaw cycles

Protect substrate from prolonged exposure to light.

Handle with care

reuse standard solution, working solution of Detection Ab and HRP Conjugate, which have been diluted. If you need to use the standard repeatedly, you can divide the standard into a small pack according to the amount of each assay, keep them at -20~-80°C and avoid repeated freezing and thawing.

6. **Reaction Time Control**: Control reaction time by following this protocol.
7. **Substrate**: Substrate Solution is easily contaminated. Protect it from light.
8. **Stop Solution**: As it is an acid solution, protect your eyes, hands, face and clothes when using this solution.
9. **Mixing**: You should use micro-oscillator at the lowest frequency, as sufficient and gentle mixing is particularly important to reaction result. If there is no micro-oscillator available, you can tap the ELISA plate frame gently with your finger before reaction.
10. **Security**: Wear lab coats and gloves for protection. Especially detecting samples of blood or other biological fluid please perform assay following the national security columns of biological laboratories.
11. Do not use components from different batches of kit (washing buffer and stop solution can be an exception).
12. To avoid cross-contamination, change pipette tips between the addition of each standard, sample, and reagent. Also, use separate reservoirs for each reagent. Otherwise, the results will be inaccurate**.**

CALCULATION OF RESULTS

Average the duplicate readings for each standard and samples, then subtract the average zero standard optical density. Create a standard curve by plotting the mean OD value 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. It is recommended to use some professional software, such as Assay Blaster!, to do this calculation. In the software interface, a best fitting equation of standard curve will be calculated using OD values and concentrations of standard sample. The software will calculate the concentration of samples after entering the OD value of samples. If samples have been diluted, the concentration calculated from the standard curve must be multiplied by the dilution factor. If the OD of the sample surpasses the upper limit of the standard curve, you should re-test it after appropriate dilution. The actual concentration is the calculated concentration multiplied by the dilution factor.

SUMMARY

1. Add 100µl standard or sample to each well. Incubate 90 minutes at 37°C
2. Remove the liquid. Add 100µl Biotinylated Detection Ab. Incubate for 1 hour at 37°C
3. Aspirate and wash 3 times
4. Add 100µl HRP Conjugate. Incubate 30 minutes at 37°C
5. Aspirate and wash 5 times
6. Add 90µl Substrate Reagent. Incubate 15 minutes at 37°C
7. Add 50µl Stop Solution. Read at 450nm immediately
8. Calculate results

TROUBLESHOOTING

Problem	Causes	Solutions
Poor standard curve	Inaccurate pipetting	Check pipettes.
	Improper standard dilution	Briefly spin the vial of standard and dissolve the powder thoroughly by a gentle mix.
	Wells not completely aspirated	Completely aspirate wells between steps.
Low signal	Too brief incubation times	Ensure sufficient incubation time.
	Incorrect assay temperature	Use recommended incubation temperature. Bring substrate to room temperature before use.
	Inadequate reagent volumes	Check pipettes and ensure correct preparation.
	Improper dilution	
Deep color but low value	Plate reader settings not optimal	Verify the wavelength and filter setting in the plate reader.
		Open the Plate Reader ahead to pre-heat
Large CV	Inaccurate pipetting	Check pipettes
High background	Concentration of detector too high	Use recommended dilution factor.
	Plate is insufficiently washed	Review the manual for proper wash. If using a plate washer, check that all ports are unobstructed.
	Contaminated wash buffer	Make fresh wash buffer
Low sensitivity	Improper storage of the ELISA kit	All the reagents should be stored according to the instructions.
	Stop solution not added	Stop solution should be added to each well before measurement.

DECLARATION

1. Limited by current conditions and scientific technology, we can't completely conduct the comprehensive identification and analysis on all the raw material provided, so there may be some qualitative and technical risks when using this kit.
2. The final experimental results will be closely related to the validity of products, operation skills of the operators and the experimental environments. Make sure that sufficient samples are available.
3. To get the best results, only use the reagents supplied by the manufacturer and strictly comply with the instructions in the description!
4. Incorrect results may be caused by incorrect reagent preparation and loading, as well as incorrect parameter setting for microplate reader. Please read the instruction carefully and adjust the instrument prior to the experiment.
5. Even the same operator might get different results in two separate experiments. In order to get better reproducible results, the operation of every step in the assay should be controlled.
6. Every kit has strictly passed QC test. However, results from end users might be inconsistent with our data due to some unexpected reasons such as transportation conditions, different lab equipment, and so on. Intra-assay variance among kits from different batches might arise from above reasons.

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