Elabscience®

7th Edition, revised in April, 2017

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Rat TGF-β2 (Transforming Growth Factor Beta 2) ELISA Kit

Catalog No: E-EL-R1015
96T

This manual must be read attentively and completely before using this product.

If you have any problems, please contact our Technical Service Center for help.

Phone: 240-252-7368(USA) 240-252-7376(USA)
Email: techsupport@elabscience.com
Website: www.elabscience.com

Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

Intended use

This ELISA kit applies to the in vitro quantitative determination of Rat TGF-β2 concentrations in serum, plasma and other biological fluids.

Specification

- Sensitivity: 9.38pg/mL.
- Detection Range: 15.63-1000pg/mL
- Specificity: This kit recognizes natural and some recombinant Rat TGF-β2. No Significant cross-reactivity or interference between Rat TGF-β2 and analogues was observed.
- Repeatability: Coefficient of variation is < 10%.

Test principle

This ELISA kit uses Sandwich-ELISA as the method. The micro ELISA plate provided in this kit has been pre-coated with an antibody specific to Rat TGF-β2. Standards or samples are added to the micro ELISA plate wells and combined with the specific antibody. Then a biotinylated detection antibody specific for Rat TGF-β2 and Avidin-Horseradish Peroxidase (HRP) conjugate are added to each micro plate well successively and incubated. Free components are washed away. The substrate solution is added to each well. Only those wells that contain Rat TGF-β2, biotinylated detection antibody and Avidin-HRP conjugate will appear blue in color. The enzyme-substrate reaction is terminated by the addition of stop solution and the color turns yellow. The optical density (OD) is measured spectrophotometrically at a wavelength of 450 nm ± 2 nm. The OD value is proportional to the concentration of Rat TGF-β2. You can calculate the concentration of Rat TGF-β2 in the samples by comparing the OD of the samples to the standard curve.

Kit components & Storage

The unopened kit can be stored at 4℃ for 1 month. If the kit is not used within 1 month, store the items separately according to the following conditions since the kit is received.

Item	Specifications	Storage
Micro ELISA Plate (Dismountable)	8 wells ×12 strips	-20℃, 6 months
Reference Standard	2 vials	
Concentrated Biotinylated Detection Ab (100×)	1 vial, 120 uL	
Concentrated HRP Conjugate (100×)	1 vial, 120 μL	-20℃(shading light), 6 months
Reference Standard & Sample Diluent	1 vial, 20 mL	4℃, 6 months
Biotinylated Detection Ab Diluent	1 vial, 10 mL	
HRP Conjugate Diluent	1 vial, 10 mL	
Concentrated Wash Buffer (25×)	1 vial, 30 mL	
Substrate Reagent	1 vial, 10 mL	4℃(shading light)
Stop Solution	1 vial, 10 mL	4℃
Plate Sealer	5 pieces	
Product Description	1 copy	
Certificate of Analysis	1 copy	

Note: All reagent bottle caps must be tightened to prevent evaporation and microbial pollution.
The volume of reagents in partial shipments is a little more than the volume marked on the label, please use in measuring instead of directly pouring.

Other supplies required

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

Note

1. Please wear lab coats and latex gloves for protection. Please perform the experiment following the national security columns of biological laboratories, especially detecting samples of blood or other body fluid.
2. The just opened ELISA Plate may appear water-like substance, which is normal and will not have any impact on the experimental results.
3. Do not reuse the diluted standard, biotinylated detection Ab working solution, concentrated HRP conjugate working solution. The unspent undiluted concentrated biotinylated detection Ab (100×) and other stock solution should be stored back according to the storage condition in the above table.
4. The microplate reader should be able to be installed with a filter that can detect the wavelength at 450±10 nm. The optical density should be within 0~3.5.
5. Do not mix or use components from other lots (except for washing buffer and stop solution).
6. Change pipette tips between adding of each standard level, between sample adding, and between reagent adding. Also, use separate reservoirs for each reagent.

Sample collection

Serum: Allow samples to clot for 2 hours at room temperature or overnight at 4℃ before centrifugation for 15 min at 1000×g at 2~8℃. Collect the supernatant to carry out the assay. Blood collection tubes should be disposable, non-endotoxin.

Plasma: Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge samples for 15 min at 1000×g at 2~8℃ within 30 min of collection. Collect the supernatant to carry out the assay. Hemolysis samples are not suitable for ELISA assay!

Cell lysates: For adherent cells, gently wash the cells with moderate amount of pre-cooled PBS and dissociate the cells by trypsin. Collect the cell suspension into the centrifugal tube and centrifuge for 5 min at 1000×g. Discard the medium and wash the cells for 3 times with pre-cooled PBS. For each 1×10^6 cells, add 150-250 μL of pre-cooled PBS to keep the cells resuspended. Repeat the freeze-thaw process for several times until the cells are lysed fully. Centrifuge for 10min at 1500×g at 4℃. Remove the cell fragments, collect the supernatant to carry out the assay. Avoid repeated freeze-thaw cycles.

Tissue homogenates: It is recommended to get detailed references from other literatures before detecting different tissue types. For general information, hemolysis blood may affect the result, so the tissues should be minced to small pieces and rinsed in ice-cold PBS (0.01M, pH=7.4) to remove excess blood thoroughly. Tissue pieces should be weighed and then homogenized in PBS (tissue weight (g): PBS (mL) volume=1:9) 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 min at 5000×g to get the supernatant.

Cell culture supernatant or other biological fluids: Centrifuge samples for 20 min at 1000×g at 2~8℃. Collect the supernatant to carry out the assay.

Sample Activation

TGF-β in biological samples usually exist in unactivated forms. So it must be activated before testing. There are 2 main ways of activation: heating activation and acid activation.

Heating activation: dilute samples with Reference Standard & Sample Diluent, dilute ratio is 1:10. Water bath at 80℃ for 8 minutes, take out and cool for 5 minutes, test within 2 hours .

Acid activation:

Serum/ Plasma: Add 20uL of 1M HCL to 40uL samples, mix well, incubate 10-30 minutes at room temperature. Neutralize the acidified sample by adding 20 μL of 1.2 M NaOH/0.5 M HEPES, mix well. At last, add 320uL Reference Standard & Sample Diluent, mix well and test.

Note: Sample is diluted 10 times.

Cell Culture Supernates: Add 20uL of 1M HCL to 100uL samples, mix well, incubate 10-30 minutes at room temperature. Neutralize the acidified sample by adding 20 μL of 1.2 M NaOH/0.5 M HEPES, mix well, add 60uL Reference Standard & Sample Diluent and test.

Note: Sample is diluted 2 times.

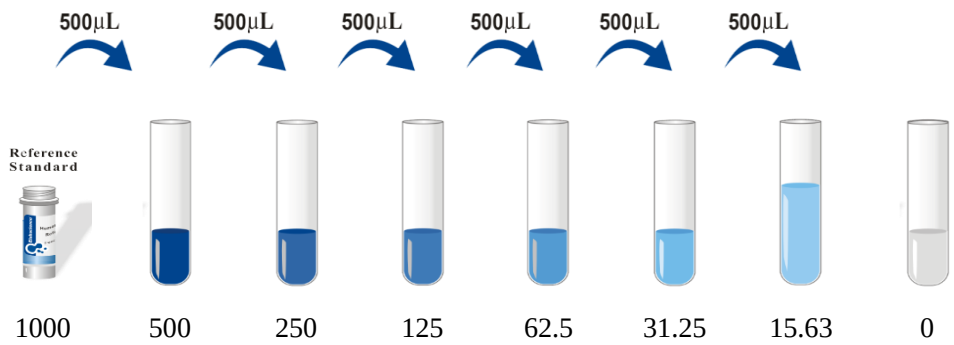
Note for sample:

1. Samples should be assayed within 7 days when stored at 4℃, otherwise samples must be divided and stored at -20℃ (≤1 month) or -80℃ (≤3 months). Avoid repeated freeze-thaw cycles.
2. Please 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.
3. If the sample type is not included in the manual, a preliminary experiment is suggested to verify the validity.
4. If lysis buffer is used to prepare tissue homogenate or cell culture supernatant, there is a possibility of causing a deviation due to the introduced chemical substance.
5. Some recombinant protein may cannot be detected due to the mismatching with coated antibody or detection antibody.

Reagent preparation

1. Bring all reagents to room temperature (18~25℃) before use. Preheat the Microplate reader for 15 min before OD measurement.
2. **Wash Buffer**: Dilute 30 mL of Concentrated Wash Buffer with deionized or distilled water to prepare 750 mL Wash Buffer. Note: if crystals have formed in the concentrate, warm it in 40℃ water bath and mix it gently until the crystals have completely dissolved.
3. **Standard working solution:** Centrifuge the standard at 10,000×g for 1 min. Add 1.0 mL of Reference Standard &Sample Diluent, let it stand for 10 min and turn it upside down for several times. After it dissolves fully, mix it thoroughly with a pipette. This reconstitution produces a working solution of 1000pg/mL. Then make serial dilutions as needed. The recommended dilution gradient is as follows: 1000、500、250、125、62.5、31.25、15.63、0 pg/mL.

   Dilution method: Take 7 EP tubes, add 500uL of Reference Standard & Sample Diluent to each tube. Pipette 500uL of the 1000pg/mL working solution to the first tube and mix up to produce a 500pg/mL working solution. Pipette 500uL of the solution from former tube to the latter one in order according to this step. The illustration below is for reference. Note: the last tube is regarded as blank. Don't pipette solution to it from the former tube.



4. **Biotinylated Detection Ab working solution:** Calculate the required amount before experiment (100 μL/well). In actual preparation, more account of 100~200μLshould be prepared. Centrifuge the stock tube before use, dilute the 100×Concentrated Biotinylated Detection Ab to 1×working solution with Biotinylated Detection Ab Diluent.
5. **Concentrated HRP Conjugate working solution:** Calculate the required amount before experiment (100 μL/well). In actual preparation, more account of 100~200 μL should be prepared. Dilute the 100× Concentrated HRP Conjugate to 1× working solution with Concentrated HRP Conjugate Diluent.

Assay procedure (A brief assay procedure is on the 11th page**)**

1. Add **Standard working solution** of different concentrations to the first two columns: Each concentration of the solution is added into two wells side by side (100 uL for each well). Add samples to other wells (100 uL for each well). Cover the plate with sealer provided in the kit. Incubate for 90 min at 37℃. Note: solutions should be added to the bottom of micro ELISA plate well, avoid touching the inside wall and foaming as possible.
2. Remove the liquid of each well, do not wash. Immediately add 100 μL of **Biotinylated Detection Ab working solution** to each well. Cover with the Plate sealer. Gently mix up. Incubate for 1 hour at 37 ℃.
3. Aspirate or decant the solution from each well， add 350 uL of **wash buffer** to each well. Soak for 1~2 min and aspirate or decant the solution from each well and pat it dry against clean absorbent paper. Repeat this wash step 3 times. Note: a microplate washer can be used in this step and other wash steps.
4. Add 100 μL of **HRP Conjugate working solution** to each well. Cover with the Plate sealer. Incubate for 30 min at 37 ℃.
5. Aspirate or decant the solution from each well, repeat the wash process for five times as conducted in step 3.
6. Add 90 μL of **Substrate Reagent** to each well. Cover with a new plate sealer. Incubate for about 15 min at 37 ℃. Protect the plate from light. Note: the reaction time can be shortened or extended according to the actual color change, but not more than 30min.
7. Add 50 μL of **Stop Solution** to each well. Note: the order to add stop solution should be the same as the substrate solution.
8. Determine the optical density (OD value) of each well at once with a micro-plate reader set to 450 nm.

Calculation of results

Average the duplicate readings for each standard and samples, then subtract the average zero standard optical density. Plot a four-parameter logistic curve on log-log graph paper, with standard concentration on the x-axis and OD values on the y-axis.

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 dilution factor.

Typical data

As the OD values of the standard curve may vary according to the conditions of actual assay performance (e.g. operator, pipetting technique, washing technique or temperature effects), the operator should establish standard curve for each test. Typical standard curve and data below is provided for reference only.

Concentration(pg/mL)	1000	500	250	125	62.5	31.25	15.63	0
OD	2.353	1.628	0.875	0.418	0.265	0.163	0.115	0.064
Corrected OD	2.289	1.564	0.811	0.354	0.201	0.099	0.051	-

Precision

Intra-assay Precision (Precision within an assay): 3 samples with low, middle and high level Rat TGF-β2 were tested 20 times on one plate, respectively.

Inter-assay Precision (Precision between assays): 3 samples with low, middle and high level Rat TGF-β2 were tested on 3 different plates, 20 replicates in each plate.

	Intra-assay Precision			Inter-assay Precision		
Sample	1	2	3	1	2	3
n	20	20	20	20	20	20
mean(pg/mL)	50.3	122.97	358.49	45.39	123.76	338.46
Standard deviation	3.28	6.84	12.48	2.84	5.59	18.24
CV (%)	6.52	5.56	3.48	6.26	4.52	5.39

Recovery

The recovery of Rat TGF-β2 spiked to three different levels in samples throughout the range of the assay in various matrices was evaluated.

Sample Type	Range (%)	Average Recovery (%)
Serum (n=5)	94-105	100
EDTA plasma (n=5)	84-99	90
Cell culture media (n=5)	92-108	99

Linearity

Samples were spiked with high concentrations of Rat TGF-β2 and diluted with Reference Standard & Sample Diluent to produce samples with values within the range of the assay.

		Serum (n=5)	EDTA plasma (n=5)	Cell culture media (n=5)
1:2	Range (%)	87-99	96-112	89-100
	Average (%)	94	104	95
1:4	Range (%)	89-103	86-96	87-101
	Average (%)	94	91	92
1:8	Range (%)	93-105	86-100	82-95
	Average (%)	99	92	87
1:16	Range (%)	88-100	86-96	82-96
	Average (%)	93	91	88

Troubleshooting

Problem	Causes	Solutions
Poor standard curve	Inaccurate pipetting	Check pipettes.
	Improper standard dilution	Ensure briefly spin the vial of standard and dissolve the powder thoroughly by gentle mixing.
	Wells are not completely aspirated	Completely aspirate wells between steps.
Low signal	Insufficient incubation time	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	
	HRP conjugate inactive or TMB failure	Mix HRP conjugate and TMB, rapid coloring.
Deep color but low value	Plate reader setting is not optimal	Verify the wavelength and filter setting in the Microplate reader.
		Open the Microplate Reader ahead to pre-heat.
Large CV	Inaccurate pipetting	Check pipettes.
High background	Concentration of target protein is 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	Prepare fresh wash buffer.
Low sensitivity	Improper storage of the ELISA kit	All the reagents should be stored according to the instructions.
	Stop solution is not added	Stop solution should be added to each well before measurement.

SUMMARY

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

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 might be some qualitative and technical risks for users using the kit.
2. The final experimental results will be closely related to the validity of products, operation skills of the operators and the experimental environments. Please make sure that sufficient samples are available.
3. To get the best results, please only use the reagents supplied by the manufacturer and strictly comply with the instructions in the description!
4. Incorrect results may occur because of wrong operations during the reagents preparation and loading, as well as incorrect parameter setting of Micro-plate 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 equipments, and so on. Intra-assay variance among kits from different batches might arise from above reasons, too.
7. Valid period: 6 months.