

Human TGF beta2 ELISA Kit

Enzyme-linked Immunosorbent Assay for quantitative detection of human TGF β 2

Catalog Number BMS254

Pub. No. MAN0016626 Rev. A.0 [30]

WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from thermofisher.com/support.

Product description

The human TGF β 2 ELISA is an enzyme-linked immunosorbent assay for the quantitative detection of human TGF β 2.

Summary

Transforming growth factor β (TGF β) belongs to a family of dimeric 25 kDa polypeptides that are ubiquitously distributed in tissues and synthesized by many different cells. Three isoforms of transforming Growth Factor β (TGF β 1, β 2 and β 3) exist in mammals. They play critical roles in growth regulation and development. Each isoform is encoded by a unique gene on different chromosomes. All three of these growth factors are secreted by most cell types, generally in a latent form, requiring activation before they can exert biological activity. The TGF- β s possess three major activities: they inhibit proliferation of most cells, but can stimulate the growth of some mesenchymal cells; they exert immunosuppressive effects and they enhance the formation of extracellular matrix. Two types of membrane receptors possessing kinase activity are involved in signal transduction. The TGF- β s are involved in wound repair processes and in starting inflammatory reaction and then in the resolution through chemotactic attraction of inflammatory cells and fibroblast.

Contrary to TGF β 1, TGF β 2 is not produced by blood platelets. TGF β 2 is a potent cytokine which has been shown to modulate embryonic development, bone formation, mammary development, wound healing, hematopoiesis, cell cycle progression and the production of the extracellular matrix. TGF β 2 – null mice were shown to exhibit perinatal mortality and a wide range of developmental defects for a single gene description which include cardiac, lung, craniofacial, limb, spinal column, eye, inner ear and urogenital defects.

For literature update refer to our website.

Principles of the test

An anti-human TGF β 2 coating antibody is adsorbed onto microwells.

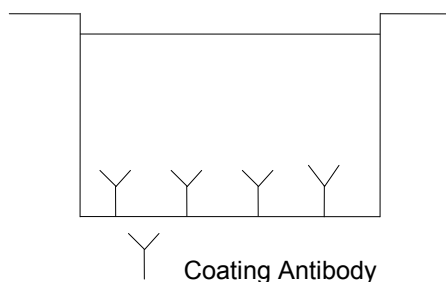


Fig. 1 Coated microwell

Human TGF β 2 present in the sample or standard binds to antibodies adsorbed to the microwells.

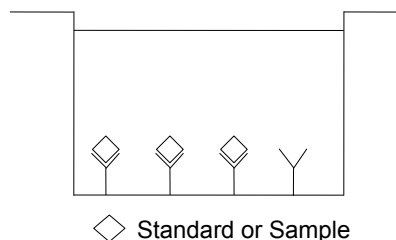


Fig. 2 First incubation

Following incubation unbound biological components are removed during a wash step. A biotin-conjugated anti-human TGF β 2 antibody is added and binds to human TGF β 2 captured by the first antibody.

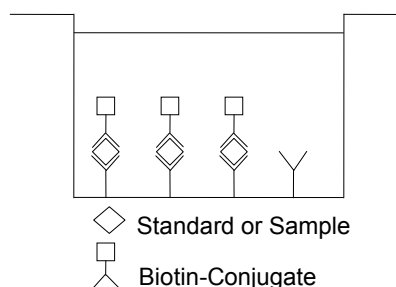


Fig. 3 Second incubation

Following incubation unbound biotin-conjugated anti-human TGF β 2 antibody is removed during a wash step. Streptavidin-HRP is added and binds to the biotin-conjugated anti-human TGF β 2 antibody.

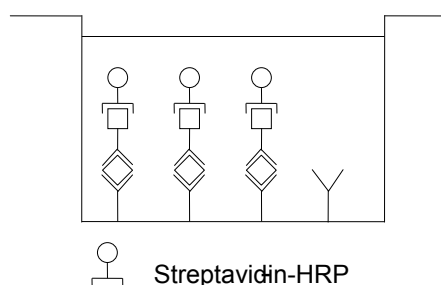


Fig. 4 Third incubation

Following incubation unbound Streptavidin-HRP is removed during a wash step, and substrate solution reactive with HRP is added to the wells.

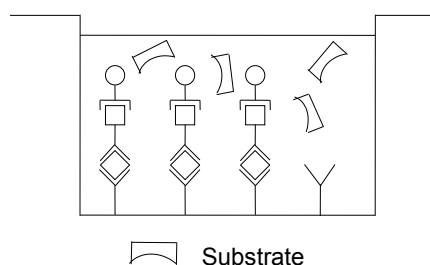


Fig. 5 Fourth incubation

A colored product is formed in proportion to the amount of human TGF β 2 present in the sample or standard. The reaction is terminated by addition of acid and absorbance is measured at 450 nm. A standard curve is prepared from 6 human TGF β 2 standard dilutions and human TGF β 2 sample concentration determined.

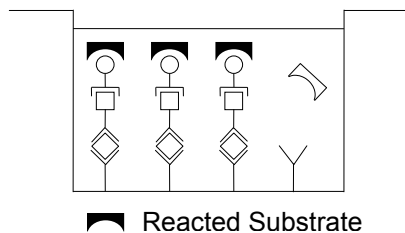


Fig. 6 Stop reaction

Reagents provided

1 aluminum pouch with a Microwell Plate (12 strips of 8 wells each) coated with monoclonal antibody to human TGF β 2

1 vial (11 mL) Biotin-Conjugate anti-human TGF β 2 monoclonal antibody

1 vial (11 mL) Streptavidin-HRP

1 vial (2 mL) human TGF β 2 Standard, 1 ng/mL

1 vial (10 mL) Assay Buffer Concentrate 10x

1 bottle (30 mL) Wash Buffer Concentrate 40x

1 vial (14 mL) Substrate Solution (tetramethyl-benzidine)

1 vial (14 mL) Stop Solution (0.5M H_2SO_4)

6 Adhesive Films

Storage instructions – ELISA kit

Store kit reagents between 2° and 8°C. Immediately after use remaining reagents should be returned to cold storage (2–8°C). Expiry of the kit and reagents is stated on labels.

Expiry of the kit components can be guaranteed only if the components are stored properly, and if, in case of repeated use of one component, this reagent is not contaminated by the first handling.

Sample collection and storage instructions

Cell culture supernatant and serum were tested with this assay. Other biological samples might be suitable for use in the assay. Remove serum from the clot as soon as possible after clotting.

Note: Pay attention to a possibly elevated blank signal in cell culture supernatant samples containing serum components (e.g., FCS), due to latent TGF β levels in animal serum.

Samples containing a visible precipitate must be clarified prior to use in the assay. Do not use grossly hemolyzed or lipemic samples.

Samples should be aliquoted and must be stored frozen at –20°C to avoid loss of bioactive human TGF β 2. If samples are to be run within 24 hours, they may be stored at 2–8°C.

Avoid repeated freeze-thaw cycles. Prior to assay, the frozen sample should be brought to room temperature slowly and mixed gently.

Materials required but not provided

- 1N NaOH and 1N HCL are needed to run the test
- 5 mL and 10 mL graduated pipettes
- 5 μ L to 1000 μ L adjustable single channel micropipettes with disposable tips
- 50 μ L to 300 μ L adjustable multichannel micropipette with disposable tips
- Multichannel micropipette reservoir
- Beakers, flasks, cylinders necessary for preparation of reagents
- Device for delivery of wash solution (multichannel wash bottle or automatic wash system)
- Microwell strip reader capable of reading at 450 nm (620 nm as optional reference wave length)

- Glass-distilled or deionized water
- Statistical calculator with program to perform regression analysis

Precautions for use

- All chemicals should be considered as potentially hazardous. We therefore recommend that this product is handled only by those persons who have been trained in laboratory techniques and that it is used in accordance with the principles of good laboratory practice. Wear suitable protective clothing such as laboratory overalls, safety glasses, and gloves. Care should be taken to avoid contact with skin or eyes. In the case of contact with skin or eyes wash immediately with water. See material safety data sheet(s) and/or safety statement(s) for specific advice.
- Reagents are intended for research use only and are not for use in diagnostic procedures.
- Do not mix or substitute reagents with those from other lots or other sources.
- Do not use kit reagents beyond expiration date on label.
- Do not expose kit reagents to strong light during storage or incubation.
- Do not pipet by mouth.
- Do not eat or smoke in areas where kit reagents or samples are handled.
- Avoid contact of skin or mucous membranes with kit reagents or samples.
- Rubber or disposable latex gloves should be worn while handling kit reagents or samples.
- Avoid contact of substrate solution with oxidizing agents and metal.
- Avoid splashing or generation of aerosols.
- To avoid microbial contamination or cross-contamination of reagents or samples that may invalidate the test, use disposable pipette tips and/or pipettes.
- Use clean, dedicated reagent trays for dispensing the conjugate and substrate reagent.
- Exposure to acid inactivates the conjugate.
- Glass-distilled water or deionized water must be used for reagent preparation.
- Substrate solution must be at room temperature prior to use.
- Decontaminate and dispose samples and all potentially contaminated materials as if they could contain infectious agents. The preferred method of decontamination is autoclaving for a minimum of 1 hour at 121.5°C.
- Liquid wastes not containing acid and neutralized waste may be mixed with sodium hypochlorite in volumes such that the final mixture contains 1.0% sodium hypochlorite. Allow 30 minutes for effective decontamination. Liquid waste containing acid must be neutralized prior to the addition of sodium hypochlorite.

Preparation of reagents

1. Buffer Concentrates should be brought to room temperature and should be diluted before starting the test procedure.
2. If crystals have formed in the Buffer Concentrates, warm them gently until they have completely dissolved.

Wash buffer (1x)

1. Pour entire contents (30 mL) of the Wash Buffer Concentrate (40x) into a clean graduated cylinder. Bring to final volume of 1200 mL with glass-distilled or deionized water.
2. Mix gently to avoid foaming.
3. Transfer to a clean wash bottle and store at 2° to 8°C. Please note that Wash Buffer (1x) is stable for 30 days.

- Wash Buffer (1x) may also be prepared as needed according to the following table:

Number of Strips	Wash Buffer Concentrate (40x) (mL)	Distilled Water (mL)
1 - 6	15	585
1 - 12	30	1170

Assay buffer (1x)

- Pour the entire contents (10 mL) of the Assay Buffer Concentrate (10x) into a clean 100 mL graduated cylinder. Bring to final volume of 100 mL with distilled water. Mix gently to avoid foaming.
- Store at 2° to 8°C. Please note that the Assay Buffer (1x) is stable for 30 days.
- Assay Buffer (1x) may also be prepared as needed according to the following table:

Number of Strips	Assay Buffer Concentrate (10x) (mL)	Distilled Water (mL)
1 - 6	5.0	45.0
1 - 12	10.0	90.0

Human TGF B 2 standard

- Label 5 tubes, one for each standard point: S2, S3, S4, S5, S6.
- Then prepare 1:2 serial dilutions for the standard curve as follows: Pipette 1 mL of Assay Buffer (1x) into each tube.
- Pipette 1 mL of undiluted standard (serves as the highest standard S1, concentration of standard 1 = 1000.0 pg/mL) into the first tube, labeled S2, and mix (concentration of standard 2 = 500.0 pg/mL).
- Pipette 1 mL of this dilution into the second tube, labeled S3, and mix thoroughly before the next transfer.
- Repeat serial dilutions 3 more times thus creating the points of the standard curve ranging from 1000.0 to 31.3 pg/mL (see Figure 7).

Assay Buffer (1x) serves as blank.

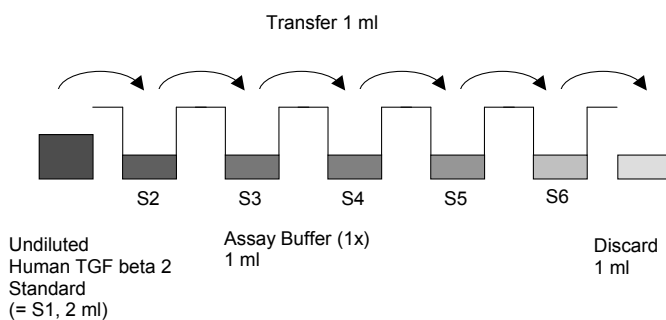


Fig. 7 Human TGF B 2 standard

Test protocol

- Predilute your samples before starting with the test procedure. Dilute serum and plasma samples 1:50 with Assay Buffer (1x) according to the following scheme:
10 µL sample + 490 µL Assay Buffer (1x)
Dilute cell culture supernatants with Assay Buffer (1x) according to the expected human TGF β 2 concentration
- Add 20 µL 1N HCl to 200 µL of prediluted samples and standards, mix and incubate for 15 minutes at room temperature. Neutralize by addition of 20 µL 1N NaOH (check pH 7-8).
- Determine the number of microwell strips required to test the desired number of samples plus appropriate number of wells needed for running blanks and standards. Each sample, standard, blank and optional control sample should be assayed in duplicate. Remove extra microwell strips from holder and store in foil bag with the desiccant provided at 2°-8°C sealed tightly.

- Add 100 µL of prepared and pretreated standard dilutions (S1 - S6) to the standard wells according to Table 1.

Table 1 Example of the arrangement of blanks, standards and samples in the microwell strips.

	1	2	3	4
A	Standard 1 1000.0 pg/mL	Standard 1 1000.0 pg/mL	Sample 2	Sample 2
B	Standard 2 500.0 pg/mL	Standard 2 500.0 pg/mL	Sample 3	Sample 3
C	Standard 3 250.0 pg/mL	Standard 3 250.0 pg/mL	Sample 4	Sample 4
D	Standard 4 125.0 pg/mL	Standard 4 125.0 pg/mL	Sample 5	Sample 5
E	Standard 5 6.25 pg/mL	Standard 5 6.25 pg/mL	Sample 6	Sample 6
F	Standard 6 31.3 pg/mL	Standard 6 31.3 pg/mL	Sample 7	Sample 7
G	Blank	Blank	Sample 8	Sample 8
H	Sample 1	Sample 1	Sample 9	Sample 9

- Add 100 µL of Assay Buffer (1x) in duplicate to the blank wells.
- Add 100 µL of each prediluted and pretreated sample in duplicate to the sample wells.
- Cover with an adhesive film and incubate at room temperature (18 to 25°C) for 3 hours on a microplate shaker. Alternatively, incubate at 2-8°C over night.
- Remove adhesive film and empty wells. Wash microwell strips 3 times with approximately 400 µL Wash Buffer per well with thorough aspiration of microwell contents between washes. Allow the Wash Buffer to sit in the wells for about 10 – 15 seconds before aspiration. Take care not to scratch the surface of the microwells.
After the last wash step, empty wells and tap microwell strips on absorbent pad or paper towel to remove excess Wash Buffer. Use the microwell strips immediately after washing. Alternatively microwell strips can be placed upside down on a wet absorbent paper for not longer than 15 minutes. Do not allow wells to dry.
- Add 100 µL of Biotin-Conjugate to all wells.
- Cover with an adhesive film and incubate at room temperature (18 to 25°C) for 2 hours.
- Remove adhesive film and empty wells. Wash microwell strips 3 times according to point 8. of the test protocol. Proceed immediately to the next step.
- Add 100 µL of Streptavidin-HRP to all wells, including the blank wells.
- Cover with an adhesive film and incubate at room temperature (18° to 25°C) for 20 minutes.
- Remove adhesive film and empty wells. Wash microwell strips 3 times according to point 8. of the test protocol. Proceed immediately to the next step.
- Pipette 100 µL of TMB Substrate Solution to all wells. (A slight blue color of the one component TMB does not interfere with the test results.)
- Incubate the microwell strips at room temperature (18° to 25°C) for about 15 minutes on a rotator. Avoid direct exposure to intense light.

The color development on the plate should be monitored and the substrate reaction stopped (see next point of this protocol) before positive wells are no longer properly recordable. Determination of the ideal time period for color development has to be done individually for each assay.

It is recommended to add the stop solution when the highest standard has developed a dark blue color. Alternatively the color development can be monitored by the ELISA reader at 620 nm. The substrate reaction should be stopped as soon as Standard 1 has reached an OD of 0.9 – 0.95.

17. Stop the enzyme reaction by quickly pipetting 50 μ L of Stop Solution into each well. It is important that the Stop Solution is spread quickly and uniformly throughout the microwells to completely inactivate the enzyme. Results must be read immediately after the Stop Solution is added or within one hour if the microwell strips are stored at 2 - 8°C in the dark.
18. Read absorbance of each microwell on a spectro-photometer using 450 nm as the primary wave length (optionally 620 nm as the reference wave length; 610 nm to 650 nm is acceptable). Blank the plate reader according to the manufacturer's instructions by using the blank wells. Determine the absorbance of both the samples and the standards.

Note: In case of incubation without shaking the obtained O.D. values may be lower than indicated below. Nevertheless the results are still valid.

Calculation of results

- Calculate the average absorbance values for each set of duplicate standards and samples. Duplicates should be within 20 percent of the mean value.
- Create a standard curve by plotting the mean absorbance for each standard concentration on the ordinate against the human TGF β 2 concentration on the abscissa. Draw a best fit curve through the points of the graph (a 5-parameter curve fit is recommended).
- To determine the concentration of circulating human TGF β 2 for each sample, first find the mean absorbance value on the ordinate and extend a horizontal line to the standard curve. At the point of intersection, extend a vertical line to the abscissa and read the corresponding human TGF β 2 concentration.
- If instructions in this protocol have been followed, serum and plasma samples have been diluted 1:50 and the concentration read from the standard curve must be multiplied by the dilution factor ($\times$ 50).
- It is suggested that each testing facility establishes a control sample of known human TGF β 2 concentration and runs this additional control with each assay. If the values obtained are not within the expected range of the control, the assay results may be invalid.
- A representative standard curve is shown in Figure 8.

Note: Do not use this standard curve to derive test results. Each laboratory must prepare a standard curve for each group of microwell strips assayed.

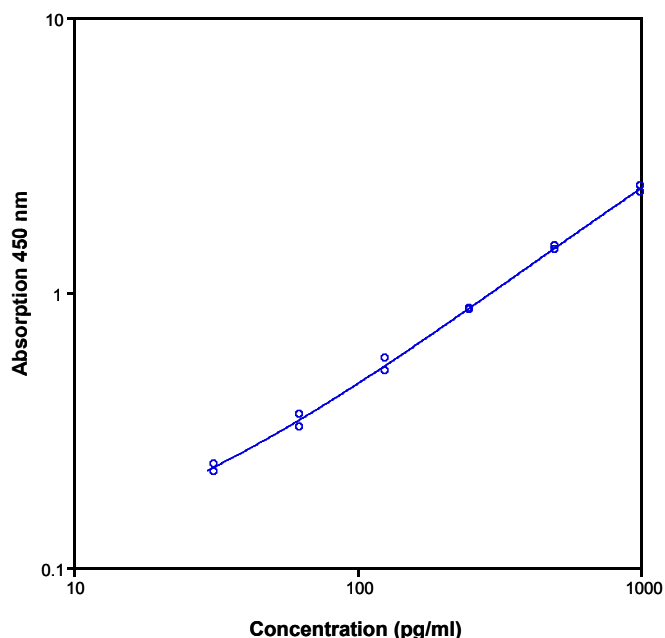


Fig. 8 Representative standard curve for human TGF β 2 ELISA. Human TGF β 2 was diluted in serial 2-fold steps in Assay Buffer (1x).

Table 2 Typical data using the human TGF β 2 ELISA.

Measuring wavelength: 450 nm

Reference wavelength: 620 nm

Standard	Human TGF β 2 Concentration (pg/mL)	O.D. at 450 nm	Mean O.D. at 450 nm	C.V. (%)
1	1000	2.323 2.448	2.386	2.6
2	500	1.431 1.483	1.457	1.8
3	250	0.874 0.870	0.872	0.2
4	125	0.521 0.576	0.548	5.0
5	63	0.325 0.360	0.342	5.0
6	31	0.224 0.237	0.231	2.8
Blank	0	0.124 0.130	0.127	2.4

The OD values of the standard curve may vary according to the conditions of assay performance (e.g., operator, pipetting technique, washing technique, or temperature effects). Furthermore, shelf life of the kit may affect enzymatic activity and thus color intensity. Values measured are still valid.

Limitations

- Since exact conditions may vary from assay to assay, a standard curve must be established for every run.
- Bacterial or fungal contamination of either screen samples or reagents or cross-contamination between reagents may cause erroneous results.
- Disposable pipette tips, flasks or glassware are preferred, reusable glassware must be washed and thoroughly rinsed of all detergents before use.
- Improper or insufficient washing at any stage of the procedure will result in either false positive or false negative results. Empty wells completely before dispensing fresh wash solution, fill with Wash Buffer as indicated for each wash cycle and do not allow wells to sit uncovered or dry for extended periods.
- The use of radioimmunotherapy has significantly increased the number of patients with human anti-mouse IgG antibodies (HAMA). HAMA may interfere with assays utilizing murine monoclonal antibodies leading to both false positive and false negative results. Serum samples containing antibodies to murine immunoglobulins can still be analyzed in such assays when murine immunoglobulins (serum, ascitic fluid, or monoclonal antibodies of irrelevant specificity) are added to the sample.

Performance characteristics

Sensitivity

The limit of detection of human TGF β 2 defined as the analyte concentration resulting in an absorbance significantly higher than that of the dilution medium (mean plus 2 standard deviations) was determined to be 6.6 pg/mL (mean of 6 independent assays).

Reproducibility

Intra-assay

Reproducibility within the assay was evaluated with 8 replicates of 3 samples containing different concentrations of human TGF β 2. The calculated overall intra-assay coefficient of variation was < 10%.

Inter-assay

Assay to assay reproducibility within one laboratory was evaluated with 12 replicates of 3 samples containing different concentrations of human TGF β 2. The calculated overall inter-assay coefficient of variation was < 10%.

Spike recovery

The spike recovery was evaluated by spiking different levels of human TGF β 2 into various samples. The overall mean recovery was >90%.

Specificity

The interference of circulating factors of the immune system was evaluated by spiking these proteins at physiologically relevant concentrations into a human TGF β 2 positive serum. No cross-reactivity was detected, notably not with human TGF β 1 and human TGF β 3.

Reagent preparation summary

Wash buffer (1x)

Add Wash Buffer Concentrate 40x (30 mL) to 1200 mL distilled water.

Number of Strips	Wash Buffer Concentrate (mL)	Distilled Water (mL)
1 - 6	15	585
1 - 12	30	1170

Assay buffer (1x)

Add Assay Buffer Concentrate 10x (10 mL) to 90 mL distilled water.

Number of Strips	Assay Buffer Concentrate (mL)	Distilled Water (mL)
1 - 6	5.0	45.0
1 - 12	10.0	90.0

Human TGF β 2 standard

Prepare serial dilutions of the concentrated human TGF β 2 standard in Assay Buffer (1x).

Test protocol summary

1. Predilute serum and plasma samples with Assay Buffer (1x) 1:50.
2. Pretreat samples and standards with HCl and NaOH.
3. Determine the number of microwell strips required.
4. Add 100 μ L of the pretreated standard dilutions in duplicate to the designated standard wells.
5. Add 100 μ L Assay Buffer (1x), in duplicate, to the blank wells.
6. Add 100 μ L prediluted and pretreated sample in duplicate to designated sample wells.
7. Cover microwell strips and incubate 3 hours at room temperature (18° to 25°C), alternatively at 2-8°C over night.

8. Empty and wash microwell strips 3 times with Wash Buffer.
9. Add 100 μ L Biotin-Conjugate to all wells.
10. Cover microwell strips and incubate 2 hours at room temperature (18° to 25°C).
11. Empty and wash microwell strips 3 times with Wash Buffer.
12. Add 100 μ L Streptavidin-HRP to all wells.
13. Cover microwell strips and incubate 20 minutes at room temperature (18° to 25°C).
14. Empty and wash microwell strips 3 times with Wash Buffer.
15. Add 100 μ L of TMB Substrate Solution to all wells.
16. Incubate the microwell strips for about 15 minutes at room temperature (18° to 25°C).
17. Add 50 μ L Stop Solution to all wells.
18. Blank microwell reader and measure color intensity at 450 nm.

Note: If instructions in this protocol have been followed, serum and plasma samples have been diluted 1:50 and the concentration read from the standard curve must be multiplied by the dilution factor

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 - Safety Data Sheets (SDSs; also known as MSDSs)

Note: For SDSs for reagents and chemicals from other manufacturers, contact the manufacturer.

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