DIACLONE
A Tepnel Company

Human TGF-β2 ELISA

650 020 096 **1 x 96 tests**
650 020 192 **2 x 96 tests**

INTENDED USE

The TGF-β2 ELISA is an enzyme-linked immunosorbent assay for the quantitative detection of human transforming growth factor beta-2 levels in cell culture supernatants, human serum, plasma, or other body fluids. **The TGF-β2 ELISA is for research use only. Not for use in diagnostic or therapeutic procedures.**

SUMMARY

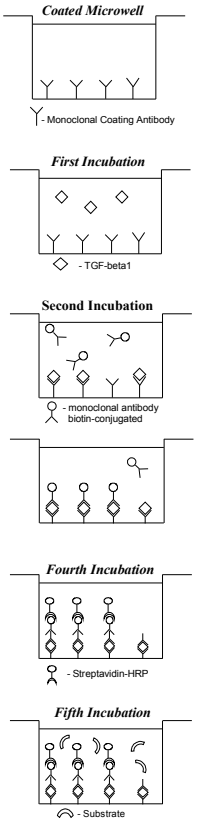
Transforming growth factor-β (TGF-β) belongs to a family of dimeric 25 kDa polypeptides that are ubiquitously distributed in tissues and synthesized by many different cells (6). Three isoforms of transforming Growth Factor-β (TGF-beta1, beta-2 and beta-3) exist in mammals. They play critical roles in 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-betas possess three major activities: they inhibit proliferation of most cells, but can stimulate the growth of some mesechymal 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-betas are involved in wound repair processes and in starting inflammatory reaction and then in the resolution through chemotactic attraction of inflammatory cells and fibroblast (7).

Contrary to TGF-β1, TGF-β2 is not produced by blood platelets.
TGF-β2 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-β2 – 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 urogenitial defects (9).
TGF-β2 has been shown to be a potent growth inhibit factor of uveal melanocytes (4). It has been described as a factor in the regulation of postnatal cerebellar neurons and neuroblast proliferation (1).

TGF-β2 has been detected in tear fluid (2). TGF-β2 levels are elevated in the vitreous of patients with proliferative diabetic retinopathy (3). Elevated plasma levels of TGF-β2 have been described in patients with disseminated malignant melanoma (5). TGF-β2 concentrations are furthermore elevated in Parkinson's disease in ventricular cerebrospinal fluid (10).

In laboratory animals TGF-β2 was shown to reduce the number of gonocytes by increasing apoptosis (8).

PRINCIPLES OF THE TEST



An anti-TGF-β2 coating antibody is adsorbed onto microwells.

TGF-β2 present in the sample or standard binds to antibodies adsorbed to the microwells. Following incubation a monoclonal anti-TGF-β2 antibody conjugated to biotin is added and binds to TGF-β2 captured by the first antibody.

Following incubation unbound monoclonal anti-TGF-β2 biotin is removed during a wash step.

Streptavidin-HRP is added and binds to the biotinylated TGFβ2. After incubation and a wash step a substrate solution reactive with HRP is added to the wells.

A coloured product is formed in proportion to the amount of TGF-β2 present in the sample. The reaction is terminated by addition of acid and absorbance is measured at 450 nm. A standard curve is prepared from six TGF-β2 standard dilutions and TGF-β2 sample concentration determined.

REAGENTS PROVIDED

REAGENTS (store at 2-8°C)	QTY 1 plate	QTY 2 plates	RECONSTITUTION
96-wells precoated microtiter plate	1	2	Ready-to-use
Plate covers	2	4	
TGF-β2 Standard: 1000 pg/ml	1 vial	2 vials	(2 ml) Make serial dilutions of the stock standard
Biotin conjugate monoclonal antibody to TGF-β2	1 vial	2 vials	(11ml) Ready -to-use
Streptavidin-HRP	1 vial	2 vials	(11 ml) Ready-to-use
1N HCl (pretreatment of samples)	1 vial	2 vials	(2 ml) Ready to use
1N NaOH (pretreatment of samples)	1 vial	2 vials	(2 ml) Ready to use
Assay Buffer Concentrate	1 bottle	2 bottles	(10 ml) 10X concentrate. Dilute in distilled water
Wash Buffer Concentrate	1 bottle	2 bottles	(30 ml) 40X concentrate. Dilute in distilled water
Substrate Solution	1 vial	2 vials	(11 ml) Ready-to-use
Stop Solution (0.5 M Phosphoric acid)	1 vial	2 vials	(6 ml) Ready-to-use

MATERIALS REQUIRED BUT NOT PROVIDED

- 5 ml and 10 ml graduated pipettes
- 5 µl to 1,000 µl adjustable single channel micropipettes with disposable tips
- 50 µl to 300 µl 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 linear regression analysis

SAFETY

- Reagents are intended for research use only and are not for use in diagnostic or therapeutic 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 pipette 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 specimens.
- Rubber or disposable latex gloves should be worn while handling kit reagents or specimens.
- Reagents containing thimerosal as preservative may be toxic if ingested.
- Avoid contact of substrate solutions with oxidizing agents and metal.
- Avoid splashing or generation of aerosols.
- In order to avoid microbial contamination or cross-contamination of reagents or specimens which may invalidate the test use disposable pipette tips and/or pipettes.
- Use clean, dedicated reagent trays for dispensing the conjugate and substrate reagents.
- Exposure to acids will inactivate the conjugate.
- Glass-distilled water or deionized water must be used for reagent preparation.
- Substrate solutions must be at room temperature prior to use.
- Decontaminate and dispose specimens 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.

STORAGE INSTRUCTIONS

Store kit reagents between 2° and 8°C. Immediately after use remaining reagents should be returned to cold storage (2° to 8°C). Expiry of the kit and reagents is stated on box front labels.
The expiry of the kit components can only be guaranteed if the components are stored properly, and if, in case of repeated use of one component, the reagent is not contaminated by the first handling.

SPECIMEN COLLECTION, PROCESSING AND STORAGE

Cell culture supernatants, murine serum, plasma or other biological samples will be suitable for use in the assay. Remove serum from the clot or red cells, respectively, as soon as possible after clotting and separation.
Samples containing a visible precipitate must be clarified prior to use in the assay. Do not use grossly hemolyzed or lipemic specimens.
Clinical samples should be kept at 2° to 8°C and separated rapidly (within half an hour) before storing at -20°C to avoid loss of bioactivity. Avoid repeated freeze-thaw cycles.

PREPARATION OF REAGENTS

1. Wash Buffer

If crystals have formed in the Wash Buffer Concentrate, warm it gently until they have completely dissolved.

Dilute the **Wash Buffer Concentrate** (40X) in a clean graduated cylinder. Mix gently to avoid foaming.

Transfer to a clean wash bottle and store at 2° to 25°C. Please note that the Wash Buffer is stable for 30 days. Wash Buffer may be prepared as needed according to the following table:

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

2. Assay Buffer

Add the content of the vial (10X) to 90 ml distilled water.

3. Preparation of Standards

Make serial dilution of the stock Standard (1000 pg/ml) in appropriate vials.

Standard 1		1000 pg/ml
Standard 2	1ml Standard 1+ 1 ml Assay Buffer	500 pg/ml
Standard 3	1ml Standard 2+ 1 ml Assay Buffer	250 pg/ml
Standard 4	1ml Standard 3+ 1 ml Assay Buffer	125 pg/ml
Standard 5	1ml Standard 4+ 1 ml Assay Buffer	62.5 pg/ml
Standard 6	1ml Standard 5+ 1 ml Assay Buffer	31.25 pg/ml

TEST PROTOCOL

a. Prepare your samples and standards before starting with the test procedure.

Dilute Standards as shown previously.

Serum and Plasma: Dilute serum and plasma samples 1:50 with Assay Buffer (490 µl diluent + 10 µl sample).

Cell Culture samples: Centrifuge and use either undiluted supernatant or dilute with Assay Buffer according to the expected TGFβ2 concentration.

Add 20 µl 1N HCl to 200 µl of prediluted sample or standards, mix and incubate for 15 minutes at room temperature. Neutralize by addition of 20 µl 1N NaOH (check pH 7-8).

b. Mix all reagents thoroughly without foaming before use.

c. 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 sufficient **Microwell Strips coated with Antibody** to human TGF-□2 from the aluminium pouch immediately prior to use. Return any wells not required for this assay with desiccant to the pouch. Seal tightly.

d. Add 100µl of each prepared standard, in duplicate, to the standard wells according to Figure 1.

Figure 1. Diagram depicting an example of the arrangement of blanks, standards and samples in the microwell strips:

	Standard Concentrations pg/mL		Sample wells									
	1	2	3	4	5	6	7	8	9	10	11	12
A	1000	1000										
B	500	500										
C	250	250										
D	125	125										
E	62.5	62.5										
F	31.25	31.25										
G	Blank	Blank										
H	Sample	Sample										

e. Add 100 µl of **Assay Buffer,** in duplicate, to the blank wells.

f. Add 100 µl of each prediluted, pretreated **Sample**, in duplicate, to the designated wells.

g. Cover with a Plate Cover and incubate at room temperature (18° to 25°C) for 3 hours on a microplate shaker set at 100 rpm. Alternatively, incubate at 2-8°C over night.

h. Wash the microwell strips three times with approximately 300 µl **Wash Buffer** per well with thorough aspiration of microwell contents between washes. Take care not to scratch the surface of the microwells.

i. After the last wash, tap microwell strips on absorbent pad or paper towel to remove excess Wash Buffer. Use the microwell strips immediately after washing or place upside down on a wet absorbent paper for not longer than 15 minutes. Do not allow wells to dry.

j. Add 100 µl of **Biotin Conjugate monoclonal TGF-□2 antibody** to each well.

k. Cover the wells and incubate the plate at room temperature (18□C to 25□C) for 2 hours.

l. Wash three times according to point h. of the test protocol.

m. Add 100 µl of **Streptavidin-HRP** solution to each well

n. Cover the wells and incubate at room temperature (18□C to 25□C) for 20 minutes.

o. Remove **Plate Cover** and empty wells. Wash microwell strips 3 times according to point h. of the test protocol. Proceed immediately to the next step.

p. Pipette 100 µl of **TMB Substrate Solution** to all wells, including the blank wells. A slight blue colour of the one component TMB does not interfer with the test results.

q. Incubate the microwell strips at room temperature (18° to 25°C) for about 10 minutes, if available on a rotator set at 100 rpm. Avoid direct exposure to intense light. The point at which the substrate reaction is stopped is often determined by the ELISA reader being used. Many ELISA readers record absorbance only up to 2.0 O.D. **The O.D. values at the plate should be monitored and the substrate reaction stopped before positive wells are no longer properly recordable.**

r. Stop the enzyme reaction by quickly pipetting 50 µl of **Stop Solution** into each well, including the blank wells. 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.

s. 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 TGF-□2 standards.

Note: In case of incubation without shaking the obained 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 per cent of the mean.

- Create a standard curve by plotting the mean absorbance for each standard concentration on the ordinate against the TGF-β2 concentration on the abscissa. Draw a best fit curve through the points of the graph.

- To determine the concentration of circulating TGF-β2 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 TGF-β2 concentration.

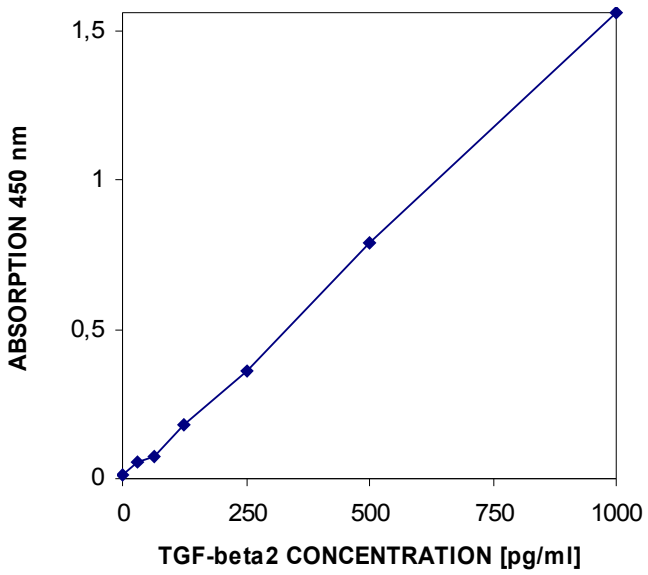
For samples which have been diluted according to the insructions given in this manual (e.g. 1:50) the concentration read from the standard curve must be multiplied by the dilution factor (e.g. x50).

Note: Calculation of samples with an O.D. exceeding the range of the standard curve may result in incorrect, low TGF-β2 levels. Such samples should be re-analyzed at higher dilution rate in order to precisely quantitate the actual TGF-β2 level.

It is suggested that each testing facility establishes a control sample of known TGF-β2 concentration and runs this additional control with each assay. If the values obtained are not within the expected range of this control, the assay results may be invalid.

- A representative standard curve is shown in Figure 3. This curve cannot be used to derive test results. Every laboratory must prepare a standard curve for each group of microwell strips assayed.

Figure 3. Representative standard curve for TGF-β2 ELISA. Recombinant soluble TGF-β2 was diluted in Assay Buffer; symbols represent the mean of three parallel titrations. Do not use this standard curve to derive test results. A standard curve must be run for each group of microwell strips assayed.



Typical data using the TGF-β2 ELISA

Measuring wavelength: 450 nm
Reference wavelength: 620 nm

Standard	TGF-β2 Concentration (pg/ml)	O.D. Mean
1	1000 1000	1.561
2	500 500	0.789
3	250 250	0.358
4	125 125	0.182
5	62.5 62.5	0.0740
6	31.3 31.3	0.0550
Blank	0 0	0.0098

LIMITATIONS OF THE PROCEDURE

- Since exact conditions may vary from assay to assay, a standard curve must be established for every run.
- Bacterial or fungal contamination of either 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. Completely empty wells before dispensing fresh Wash Buffer, fill with Wash Buffer as indicated for each wash cycle and do not allow wells to sit uncovered or dry for extended periods.

PERFORMANCE CHARACTERISTICS

Sensitivity

The limit of detection for recombinant TGF-β2 spiked into normal human serum, defined as the analyte concentration resulting in an absorption significantly higher than the absorption of the dilution medium (mean plus three standard deviations) was determined to be 6.6 pg/ml (mean of three independent assays).

Reproducibility

a. Intra-assay

Intra-assay variability was determined by 8 replicates of 3 control samples. The average coefficient of variation was <10%.

b. Inter-assay

Inter-assay variability was determined by 12 replicates of 3 control samples. The average coefficient of variation was <10%.

Specificity

The ELISA was shown to specifically detect human TGF-β2. No cross reactivity was apparent with human serum factors spiked into human serum, notably there was no cross reactivity with human TGF-β1 and TGF-β3.

Spike Recovery

Spike recovery was determined to be >90% in average of recombinant TGF-β2 spiked into various samples.

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REAGENT PREPARATION SUMMARY

A. Wash Buffer	Add 30 ml **Wash Buffer Concentrate** (40X) to 1200 ml distilled water.
B. Assay Buffer	Add 10 ml **Assay Buffer** (10X) to 90 ml distilled water
C. Standard	Make serial dilutions of the stock **standard**

TEST PROTOCOL SUMMARY

- Add 100 µl of prepared **Standard**, in duplicate, to standard wells.
- Add 100 µl **Assay Buffer**, in duplicate, to the blank wells.
- Add 100 µl prediluted, pretreated **Sample**, in duplicate, to designated wells.
- Cover microwell strips and incubate 3 hours at room temperature (18° to 25°C), alternatively over night at 2 - 8°C.
- Empty and wash microwell strips 3 times with **Wash Buffer**
- Add 100 µl of Biotin- Conjugate **monoclonal antibody** to TGF-□2 to all wells
- Incubate 2 hours covered at room temperature (18° to 25°C).
- Empty and wash microwell strips 3 times with **Wash Buffer**
- Add 100 µl of **Streptavidin-HRP conjugate**
- Incubate 20 minutes covered at room temperature (18□C to 25□C).
- Empty and wash microwell strips 3 times with **Wash Buffer**
- Add 100 µl of **TMB Substrate Solution** to all wells including blank wells.
- Incubate the microwell strips for about 10 minutes at room temperature (18° to 25°C).
- Add 50 µl **Stop Solution** to all wells including blank wells.
- Blank microwell reader and measure colour intensity at 450 nm.

Note: Calculation of samples with an O.D. exceeding the range of the standard curve may result in incorrect, low TGF-β2 levels. Such samples require further dilution with Assay Buffer in order to precisely quantitate the actual TGF-β2 level.