DRG® TGF-ß1 ELISA (EIA-1864)

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Introduction

Intended Use

The **DRG TGF-β1 ELISA** is an enzyme immunoassay for the quantitative *in vitro diagnostic* measurement of TGF-β1 in serum, plasma and cell culture supernatant.

Summary and Explanation

Transforming Growth Factor β1 (TGF-β1) is a 25 kDa Homodimer composed of two 12.5 kDa subunits joined by disulfide bonds (1). TGF-β1 is a multipotent Cytokine with cell- and dose-dependent activities. This molecule is produced by a number of cells and tissue types, e.g. thrombocytes, bone tissue, placenta and kidneys. This potent Cytokine modulates embryonic development, bone formation, mammary development, wound healing, hematopoiesis, cell cycle progression and the production of the extracellular matrix. With respect to the immune system, TGF-β1 inhibits T and B cell proliferation and acts as an anti-inflammatory molecule both in vitro and in vivo. TGF-β1 inhibits macrophage maturation and activation. This molecule also inhibits the activity of natural killer cells and lymphokine activated killer cells and blocks cytokine production.

PRINCIPLE of the test

The DRG TGF-β1 ELISA Kit is a solid phase enzyme-linked immunosorbent assay (ELISA) based on the sandwich principle.
Prior to testing the standards and patient samples are diluted in assay buffer, acidified with HCl and then neutralized with NaOH.

Afterwards, the neutralized standards and samples are added to the antibody coated (polyclonal) microtiter wells. After the first incubation the unbound sample material is removed by washing. Then a monoclonal mouse anti TGF-β1 antibody, a biotinilated anti mouse IgG antibody and the Streptavidin-HRP Enzyme complex are incubated in succession. An immuno enzyme sandwich complex is formed.
After incubation the unbound conjugate is washed off. Having added the substrate solution, the intensity of colour developed is proportional to the concentration of TGF β1 in the patient sample.

Warnings and Precautions

1. This kit is for in vitro diagnostic use only. For professional use only.
2. All reagents of this test kit which contain human serum or plasma have been tested and confirmed negative for HIV I/II, HBsAg and HCV by FDA approved procedures. All reagents, however, should be treated as potential biohazards in use and for disposal.
3. Before starting the assay, read the instructions completely and carefully. Use the valid version of the package insert provided with the kit. Be sure that everything is understood.
4. The microplate contains snap-off strips. Unused wells must be stored at 2 °C to 8 °C in the sealed foil pouch and used in the frame provided.
5. Pipetting of samples and reagents must be done as quickly as possible and in the same sequence for each step.

6. Use reservoirs only for single reagents. This especially applies to the substrate reservoirs. Using a reservoir for dispensing a substrate solution that had previously been used for the conjugate solution may turn solution colored. Do not pour reagents back into vials as reagent contamination may occur.
7. Mix the contents of the microplate wells thoroughly to ensure good test results. Do not reuse microwells.
8. Do not let wells dry during assay; add reagents immediately after completing the rinsing steps.
9. Allow the reagents to reach room temperature (21-26°C) before starting the test. Temperature will affect the absorbance readings of the assay. However, values for the patient samples will not be affected.
10. Never pipet by mouth and avoid contact of reagents and specimens with skin and mucous membranes.
11. Do not smoke, eat, drink or apply cosmetics in areas where specimens or kit reagents are handled.
12. Wear disposable latex gloves when handling specimens and reagents. Microbial contamination of reagents or specimens may give false results.
13. Handling should be done in accordance with the procedures defined by an appropriate national biohazard safety guideline or regulation.
14. Do not use reagents beyond expiry date as shown on the kit labels.
15. All indicated volumes have to be performed according to the protocol. Optimal test results are only obtained when using calibrated pipettes and microtiterplate readers.
16. Do not mix or use components from kits with different lot numbers. It is advised not to exchange wells of different plates even of the same lot. The kits may have been shipped or stored under different conditions and the binding characteristics of the plates may result slightly different.
17. Avoid contact with *Stop Solution* containing 0.5 M H_2SO_4. It may cause skin irritation and burns.
18. Some reagents contain Proclin 300, BND and/or MIT as preservatives. In case of contact with eyes or skin, flush immediately with water.
19. TMB substrate has an irritant effect on skin and mucosa. In case of possible contact, wash eyes with an abundant volume of water and skin with soap and abundant water. Wash contaminated objects before reusing them. If inhaled, take the person to open air.
20. Chemicals and prepared or used reagents have to be treated as hazardous waste according to the national biohazard safety guideline or regulation.
21. For information on hazardous substances included in the kit please refer to Material Safety Data Sheets. Material Safety Data Sheets for this product are available upon request directly from DRG.

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Reagents

Reagents provided

1. ***Microtiterwells***, 12x8 (break apart) strips, 96 wells;
   Wells coated with anti-TGF-β1 antibody (polyclonal).
2. ***Standard (Stock Standard)*****,** 1 vial, 2 mL,
   Concentrations: 600 pg/mL
   see „Preparation of Reagents".
   Contain non-mercury preservative.
3. ***Assay Buffer***, ***10X*** **concentrate,** 1 vial, 10 mL,
   Concentrations: 0 pg/mL
   see „Preparation of Reagents".
   Contains non-mercury preservative.
4. ***Antiserum***, 1 vial, 11 mL, ready to use,
   monoclonal Mouse anti-TGF-β1
   Contains non-mercury preservative.
5. ***Enzyme Conjugate***, 1 vial, 11 mL, ready to use,
   anti Mouse IgG conjugated to Biotin.
   Contains non-mercury preservative.
6. ***Enzyme Complex***, 1 vial, 11 mL, ready to use
   Streptavidin Peroxidase
   Contains non-mercury preservative.
7. ***Substrate Solution***, 1 vial, 14 mL, ready to use,
   Tetramethylbenzidine (TMB).
8. ***Stop Solution***, 1 vial, 14 mL, ready to use,
   contains 0.5M H_2SO_4,
   Avoid contact with the stop solution. It may cause skin irritations and burns.
9. ***Wash Solution***, 1 vial, 30 mL (40X concentrated),
   see „Preparation of Reagents".
10. 1 N **HCl,** 1 vial, 3 mL, ready to use,
    for acidification of the samples.
11. 1 N **NaOH**, 1 vial, 3 mL, ready to use,
    for neutralization.

Note: Additional *Assay Buffer* for sample dilution is available upon request.

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Materials required but not provided

- 1,5 mL-Reaction Caps (e.g. from Eppendorf) for sample preparation (acidification and neutralization).
- A microtiter plate calibrated reader (450 ± 10 nm) (e.g. the DRG Instruments Microtiter Plate Reader).
- Calibrated variable precision micropipettes.
- Absorbent paper.
- Distilled or deionized water
- Universal indicator paper.
- Timer
- Semi logarithmic graph paper or software for data reduction

Storage Conditions

When stored at 2-8°C unopened reagents, except the *Stock Standard*, will retain reactivity until expiration date. Do not use reagents beyond this date.
Immediately after receipt the *Stock Standard* has to be stored frozen at -20°C.

Opened reagents must be stored at 2-8°C, or as described in chapter 4.4.
Microtiter wells must be stored at 2-8°C. Once the foil bag has been opened, care should be taken to close it tightly again.
Opened kits retain activity for 6 weeks if stored as described above.

Reagent Preparation

Bring all reagents and required number of strips to room temperature prior to use.

Assay Buffer

Dilute 10 mL of concentrated *Assay Buffer* with 90 mL deionized water to a final volume of 100 mL Working Assay Buffer

Standards

Serial Dilution of the ***Stock Standard*** (600 pg/mL):

Description		Concentration
Standard A		**600 pg/mL**
Standard B	1 mL Standard A + 1 mL Assay Buffer	**300 pg/mL**
Standard C	1 mL Standard B + 1 mL Assay Buffer	**150 pg/mL**
Standard D	1 mL Standard C + 1 mL Assay Buffer	**75 pg/mL**
Standard E	1 mL Standard D + 1 mL Assay Buffer	**38 pg/mL**
Standard F	1 mL Standard E + 1 mL Assay Buffer	**19 pg/mL**
Standard G	2 mL Assay Buffer	**0 pg/mL**

Note: *The diluted standards are stable for 1 week at 2-8°C. For longer storage freeze at -20°C.*

Wash Solution

Add deionized water to the 40X concentrated Wash Solution.
Dilute 30 mL of concentrated *Wash Solution* with 1170 mL deionized water to a final volume of 1200 mL.
The diluted Wash Solution is stable for 2 weeks at room temperature.

Disposal of the Kit

The disposal of the kit must be made according to the national regulations. Special information for this product is given in the Material Safety Data Sheet.

Damaged Test Kits

In case of any severe damage to the test kit or components, DRG has to be informed in writing, at the latest, one week after receiving the kit. Severely damaged single components should not be used for a test run. They have to be stored until a final solution has been found. After this, they should be disposed according to the official regulations.

SPECIMEN Collection and Preparation

Serum or plasma (EDTA- or citrate plasma) and cell culture supernatant can be used in this assay.
Do not use haemolytic, icteric or lipaemic specimens.
Please note: Samples containing sodium azide should not be used in the assay.

Specimen Collection

Serum:

Collect blood by venipuncture (e.g. Sarstedt Monovette # 02.1388.001), allow to clot, and separate serum by centrifugation at room temperature. Do not centrifuge before complete clotting has occurred. Patients receiving anticoagulant therapy may require increased clotting time.

Plasma:

Whole blood should be collected into centrifuge tubes containing anti coagulant and centrifuged immediately after collection.
(E.g. for EDTA plasma Sarstedt Monovette – red cap - # 02.166.001;
for Citrate plasma Sarstedt Monovette – green cap - # 02.167.001.)

Specimen Storage and Preparation

Specimens should be capped and may be stored for up to 24 hours at 2 °C to 8 °C prior to assaying.
Specimens held for a longer time should be frozen only once at -20°C prior to assay. Thawed samples should be inverted several times prior to testing.

Specimen Dilution

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SERUM AND PLASMA

Serum and Plasma Samples should be diluted **1:50** with *Assay Buffer* prior to testing.
Please note: The results have to be multiplied with the dilution factor (x 50).
Example:
dilution 1:50: 10 µL Serum + 490 µL *Assay Buffer* (mix thoroughly)

CELL CULTURE SAMPLES

Centrifuge the Cell Culture Samples. Dilute the supernatant with *Assay Buffer*, according to the expected TGF-β1 concentrations, e.g. 1:10, if a high TGF-β1 concentration is expected. The results have to be multiplied with the dilution factor.
Example:
dilution 1:10: 10 µL Sample + 90 µL *Assay Buffer* (mix thoroughly)

If in an initial assay, a specimen is found to contain more than the highest standard, the specimens can be further diluted with *Assay Buffer* and reassayed as described in Assay Procedure.
For the calculation of the concentrations this additional dilution factor has to be taken into account.

Acidification and Neutralization of Samples and Standards

1. Add 200 µL Standards or **prediluted** Sample into Reaction Caps (e.g. Eppendorf-Caps).
   Please note: The standards, which have been prepared by serial dilution of the stock standard should also be prepared as described below.
2. Add 20 µL 1 N HCl to all caps
3. Close cups, mix thoroughly (vortex) and let stand for 15 minutes
4. Add 20 µL 1N NaOH for neutralization and mix thoroughly.
5. After neutralization the sample should have a pH value between 7 and 8. Therefore please check the pH value with Universal Indicator Paper!

Assay procedure

General Remarks

- All reagents and specimens must be allowed to come to room temperature before use. All reagents must be mixed without foaming.
- Once the test has been started, all steps should be completed without interruption.
- Use new disposal plastic pipette tips for each standard, control or sample in order to avoid cross contamination.
- Absorbance is a function of the incubation time and temperature. Before starting the assay, it is recommended that all reagents are ready, caps removed, all needed wells secured in holder, etc. This will ensure equal elapsed time for each pipetting step without interruption.
- As a general rule the enzymatic reaction is linearly proportional to time and temperature.

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Test Procedure

Each run must include a standard curve.

1. Secure the desired number of Microtiter wells in the frame holder.
2. Dispense **100 µL** of each pretreated ***Standard, Control*** and **samples** with new disposable tips into appropriate wells. (Please refer to chapters "Specimen Dilution" and "Acidification and Neutralization of Samples and Standards".)
3. Cover the plate and incubate **over night (8 - 16 hours)** at 4°C.
   Alternative: 3 hours incubation at room temperature.
4. Briskly shake out the contents of the wells. Rinse the wells **3 times** with diluted wash solution, 300 µl per Well. Strike the wells sharply on absorbance paper to remove residual droplets.
   Important note: The sensitivity and precision of this assay is markedly influenced by the correct performance of the washing procedure!
5. Dispense **100 µL** *Antiserum* into all wells.
6. Incubate **120 minutes** at room temperature.
7. Briskly shake out the contents of the wells. Rinse the wells **3 times** with diluted wash solution, 300 µl per Well. Strike the wells sharply on absorbance paper to remove residual droplets.
8. Dispense **100 µl** *Enzyme Conjugate* (Anti Mouse Biotin) into each well.
9. Incubate **45 minutes** at room temperature.
10. Briskly shake out the contents of the wells. Rinse the wells **3 times** with diluted wash solution, 300 µl per Well. Strike the wells sharply on absorbance paper to remove residual droplets.
11. Dispense **100 µl** *Enzyme Complex* into each well.
12. Incubate **45 minutes** at room temperature.
13. Briskly shake out the contents of the wells. Rinse the wells **3 times** with diluted wash solution, 300 µl per Well. Strike the wells sharply on absorbance paper to remove residual droplets.
14. Add **100 µL** of ***Substrate Solution*** to each well.
15. Incubate for **15 minutes** at room temperature.
16. Stop the enzymatic reaction by adding **50 µL** of ***Stop Solution*** to each well.
17. Determine the absorbance (OD) of each well at **450 ± 10 nm** with a microtiter plate reader.
    It is recommended that the wells be read **within 10 minutes** after adding the *Stop Solution*.

Calculation of Results

1. Calculate the average absorbance values for each set of standards, controls and patient samples.
2. Using semi-logarithmic graph paper, construct a standard curve by plotting the mean absorbance obtained from each standard against its concentration with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis.
3. Using the mean absorbance value for each sample determine the corresponding concentration from the standard curve.
4. Automated method: The results in the IFU have been calculated automatically using a 4 PL (4 Parameter Logistics) curve fit. 4 Parameter Logistics is the preferred method. Other data reduction functions may give slightly different results.
5. The concentration of the samples can be read directly from this standard curve. Multiply the results by the initial dilution factor (for serum serum and plasma samples by 50)
   Samples with concentrations higher than that of the highest standard have to be further diluted or reported as such. For the calculation of the concentrations this dilution factor has to be taken into account.

Expected Normal VAlues

It is strongly recommended that each laboratory should determine its own normal and abnormal values.

Data can be obtained by request.

Quality Control

Good laboratory practice requires that controls be run with each calibration curve. A statistically significant number of controls should be assayed to establish mean values and acceptable ranges to assure proper performance.
It is recommended to use control samples according to state and federal regulations. The use of control samples is advised to assure the day to day validity of results. Use controls at both normal and pathological levels.
The controls and the corresponding results of the QC-Laboratory are stated in the QC certificate added to the kit. The values and ranges stated on the QC sheet always refer to the current kit lot and should be used for direct comparison of the results.
Employ appropriate statistical methods for analysing control values and trends. If the results of the assay do not fit to the established acceptable ranges of control materials patient results should be considered invalid.
In this case, please check the following technical areas: Pipetting and timing devices; photometer, expiration dates of reagents, storage and incubation conditions, aspiration and washing methods.
After checking the above mentioned items without finding any error contact your distributor or DRG directly.

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Performance Characteristics

Assay Dynamic Range

The range of the assay is between 1.9 – 600 pg/mL

Specificity of Antibodies (Cross Reactivity)

The following substances were tested for cross reactivity of the assay:

Component	Cross reactivity
TGF-β2	none
TGF-β3	none
TGF-β1 (rat)	98%

Sensitivity

The analytical sensitivity of the DRG ELISA was calculated by adding 2 standard deviations to the mean of 20 replicate analyses of the *Standard 0* and was found to be 1.9 pg/mL

Reproducibility

Serum	n	CV %
Intra assay	8	1.0
Inter assay	12	7.5

Recovery

Recovery: 92,5 % (n=2).
Ca. 93% of the original sample are found in the recovery test.

Linearity

Plasma samples were diluted with Assay Buffer.

Dilution	measured Concentration
	[pg/ml]
1:30	48,73
1:40	55,19
1:50	56,33
1:60	58,00

Limitations of Use

Reliable and reproducible results will be obtained when the assay procedure is performed with a complete understanding of the package insert instruction and with adherence to good laboratory practice.
Any improper handling of samples or modification of this test might influence the results.

Interfering Substances

Data can be obtained on request.

Drug Interferences

Until today no substances (drugs) are known to us, which have an influence to the measurement of TGF-β1 in a sample.

High-Dose-Hook Effect

Data can be obtained on request.

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Legal Aspects

Reliability of Results

The test must be performed exactly as per the manufacturer's instructions for use. Moreover the user must strictly adhere to the rules of GLP (Good Laboratory Practice) or other applicable national standards and/or laws. This is especially relevant for the use of control reagents. It is important to always include, within the test procedure, a sufficient number of controls for validating the accuracy and precision of the test.
The test results are valid only if all controls are within the specified ranges and if all other test parameters are also within the given assay specifications. In case of any doubt or concern please contact DRG.

Therapeutic Consequences

Therapeutic consequences should never be based on laboratory results alone even if all test results are in agreement with the items as stated under point 11.1. Any laboratory result is only a part of the total clinical picture of a patient.
Only in cases where the laboratory results are in acceptable agreement with the overall clinical picture of the patient should therapeutic consequences be derived.
The test result itself should never be the sole determinant for deriving any therapeutic consequences.

Liability

Any modification of the test kit and/or exchange or mixture of any components of different lots from one test kit to another could negatively affect the intended results and validity of the overall test. Such modification and/or exchanges invalidate any claim for replacement.
Claims submitted due to customer misinterpretation of laboratory results subject to point 11.2. are also invalid. Regardless, in the event of any claim, the manufacturer's liability is not to exceed the value of the test kit. Any damage caused to the test kit during transportation is not subject to the liability of the manufacturer.

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