ELISA kits available from ADI (see details at the web site)

#0010 Human Leptin
#200-120-AGH Human globular Adiponectin (gAcrp30)
#0700 Human Sex Hormone Binding Glob (SHBG)
#0900 Human IGF-Binding Protein 1 (IGFBP1)
#1000 Human C-Reactive Protein (CRP)
#100-110-RSH Human Resistin /FIZZ3
#100-140-ADH Human Adiponectin (Acrp30)
#100-160-ANH Human Angiogenin
#100-180-APH Human Angiopoietin-2 (Ang-2)
#100-190-B7H Human Bone Morphogenic Protein 7 (BMP-7)
#1190 Human Serum Albumin **#1200** Human Albumin (Urinary)
#1750 Human IgG (total) **#1760** Human IgM
#1800 Human IgE **#1810** Human Ferritin
#1210 Human Transferrin (Tf)
#0020 Beta-2 microglobulin
#1600 Human Growth Hormone (GH)
#0060 Human Pancreatic Colorectal cancer (CA-242)
#1820 Human Ovarian Cancer (CA125) **#1830** Human CA153
#1840 Human Pancreatic & GI Cancer (CA199)
#1310 Human Pancreatic Lipase
#1400 Human Prostatic Acid Phosphatase (PAP)
#1500 Human Prostate Specific Antigen (PSA) **#1510** free PSA (fPSA)
#0500 Human Alpha Fetoprotein (AFP)
#0050 Human Neuron Specific Enolase (NSE)

#0030 Human Insulin **#0040** Human C-peptide
#0100 Human Luteinizing Hormone (LH)
#0200 Human Follicle Stimulating Hormone (FSH)
#0300 Human Prolactin (PRL)
#0400 Human Chorionic Gonadotropin (HCG) #**0410** HCG-free beta

#0600 Human Thyroid Stimulating Hormone (TSH)
#1100 Human Total Thyroxine (T4) **#1110** Human Free T4 (fT4)
#1650 Human free triiodothyronine (fT3) **#1700** Human T3 (total)

#1850 Human Cortisol **#1860** Human Progesterone
#1865 Human Pregnolone **#1875** Human Aldosterone
#1880 Human Testosterone **#1885** Human free Testosterone
#1910 Human Androstenedione **#1920** Human Estradiol
#1925 Human Estrone **#1940** Dihydrotestosterone (DHT)
#1950 Human DHEA-sulphate (DHEA-S)
#3400 Human serum Neopterin

#3000 Human Rheumatoid Factors IgM (RF)
#3100 Human anti-dsDNA
#3200 Anti-Nuclear Antibodies (ANA)

Instruction Manual No. M- MTGF-105

Mouse Transforming Growth Factor beta (TGFB) ELISA Kit 96 tests, Quantitative

Cat # MTGF-105, 96 Tests

For Quantitative Determination of TGFB in serum, plasma

ALPHA DIAGNOSTIC INTERNATIONAL

4638 N Loop • San Antonio• Texas 78244 • USA.
Phone (210) 561-9515 • Fax (210) 561-9544
Toll Free (800) 786-5777

Email: service@4adi.com
Web: www.4adi.com

Mouse TGFB ELISA KIT # MTGF-105 Kit Contents (96 tests):

Components	#
Anti- TGFB coated microwell strips (96 wells, 12 x 8 wells), # MTGF-105P	1 Plate
Standard Lyophilized, 2000 pg # MTGF-106	2 vials
Sample / Standard **dilution buffer**, # MTGF-107, **20 ml**	1 bottle
Biotin-detection antibody (Concentrated), # MTGF-108, **120 ul**	1 vial
Antibody **dilution buffer**, # MTGF-109, **10 ml**	1 bottle
HRP-Streptavidin Conjugate, # sHRP-105, **120 ul**	1 vial
HRP-Streptavidin Conjugate **dilution buffer**, # SCDB-105, **10 ml**	1 bottle
TMB Substrate, # MTGF-105-TM, **10 ml**	1 bottle
Stop Solution, # MTGF-105-ST, **10 ml**	1 bottle
Wash buffer (25X), # MTGF-105-WB, **30 ml**	1 bottle
Preprocess Agent A, # MTGF-105-PA**, 1.5 ml**	2 Vials
Preprocess Agent B, # MTGF-105-PB**, 1.5 ml**	2 Vials
Complete Instruction **Manual,** M- MTGF-105	1

Intended Use

ADI's Mouse TGFB ELISA kit is a highly sensitive sandwich type assay for the measurement of TGFB in serum, plasma, tissue homogenates and other biological fluids. **For research use only (RUO)**, not for diagnostic procedures.

General Information

TGF beta (TGFB, Transforming Growth Factor Beta) is a polypeptide member of the transforming growth factor beta superfamily of cytokines, found almost ubiquitously in tissues. Transforming growth factor (TGF)-b is stored in the extracellular matrix as a latent complex with its pro-domain. Activation of TGF beta requires the binding of aV integrin to an RGD sequence in the pro-domain and exertion of force on this domain, which is held in the extracellular matrix by latent TGF-b binding proteins. Latent forms are complexes of TGF beta, an amino-terminal portion of the TGF-beta precursor, designated TGF-LAP (TGF-latency associated peptide), and a specific binding protein, known as LTBP. TGF beta helps regulates proliferation, differentiation, adhesion, migration in many cell types. Many cells have TGF beta receptors, and the protein positively and negatively regulates many other growth factors. TGF beta is cleaved into a latency-associated peptide and a mature TGF beta peptide, and is found in either a latent form composed of a TGFB homodimer, a LAP homodimer, and a latent TGFB binding protein, or in an active form composed of a TGF beta homodimer. The mature peptide may also form heterodimers with other TGF beta family members. The gene for TGF beta is frequently upregulated in tumor cells, and mutations in this gene result in Camurati-Engelmann disease and cystic fibrosis.

For valid test results, adequate controls and other parameters must be within the listed ranges and assay requirements.

3. If test kits are altered, such as by mixing parts of different kits, which could produce false test results, or if results are incorrectly interpreted, ADI shall have no liability.

4. If computer controlled data reduction is used to interpret the results of the test, it is imperative that the predicted values for the calibrators fall within 10% of the assigned concentrations.

PERFORMANCE CHARACTERISTICS

Precision

The within and between assay precision of the TGFB ELISA Test System were determined by analyses on three 3 samples with low, middle and high level TGFB.

CV (%) = SD/meanX100
Intra-Assay: CV<8%
Inter-Assay: CV<10%

Sensitivity

The TGFB ELISA Test System has a sensitivity of 18.75 pg/ml.

Detection Range

31.25-2000 pg/ml

Recovery

Matrices listed below were spiked with certain level of TGFB and the recovery rates were calculated by comparing measured value to the expected amount of TGFB in samples.

Matrix	Recovery range (%)	Average (%)
Serum (n=5)	90-101	94
EDTA plasma (n=5)	96-103	100
Heparin plasma (n=5)	91-100	95

Linearity

The linearity of the kit was assayed by testing samples spiked with appropriate concentration of TGFB and their serial dilutions. The results were demonstrated by the percentage of calculated concentration to the expected.

Sample	1:2	1:4	1:8
Serum (n=5)	87-102%	86-100%	92-105%
EDTA plasma (n=5)	85-97%	84-96%	84-98%
Heparin plasma (n=5)	81-98%	81-94%	85-99%

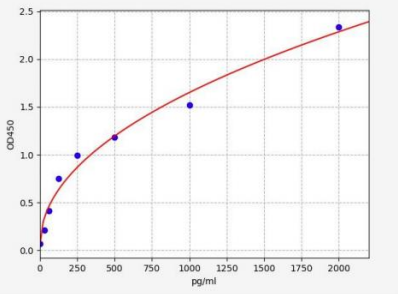
Specificity

This assay has high sensitivity and excellent specificity for detection of TGFB. No significant cross reactivity or interference between TGFB and analogues was observed.

WORKSHEET OF TYPICAL ASSAY

Wells	Stds/samples	Mean A450nm
A1, A2	Std. A (0 pg/ml)	0.079
B1, B2	Std. B (31.25 pg/ml)	0.228
C1, C2	Std. C (62.5 pg/ml)	0.394
D1, D2	Std. D (125 pg/ml)	0.64
E1, E2	Std. E (250 pg/ml)	1.154
F1, F2	Std. F (500 pg/ml)	1.647
G1, G2	Std. G (1000 pg/ml)	2.11
H1, H2	Std. H (2000 pg/ml)	2.616

NOTE: These data are for demonstration purpose only. A complete standard curve must be run in every assay to determine sample values. Each laboratory should determine their own normal reference values.



Calculation of results:

A dose response curve is used to ascertain the concentration of TGFB in unknown specimens.

1. Record the A450 obtained from the printout of the microplate reader as outlined in Example 1.
2. Plot the A450 for mean duplicate calibrator versus the TGFB concentration in pg/ml on linear graph paper. Connect the points with a best-fit curve.
3. To determine the concentration of TGFB for an unknown, locate the average A450 of the duplicates for each unknown on the vertical axis of the graph, find the intersecting point on the curve, and read the concentration (in pg/ml) from the horizontal axis of the graph (the duplicates of the unknown may be averaged as indicated).

   Note: If the samples measured were diluted, multiply the dilution factor to the concentrations from interpolation to obtain the concentration before dilution.

Interpretation

1. *Measurements and interpretation of results must be performed by a skilled individual or trained professional.*
2. Laboratory results alone are only one aspect for determining patient care and should not be the sole basis for therapy, particularly if the results conflict with other determinants.
   The reagents for the test system procedure have been formulated to eliminate maximal interference; however, potential interaction between rare serum specimens and test reagents can cause erroneous results. Heterophilic antibodies often cause these interactions and have been known to be problems for all kinds of immunoassays. For diagnostic purposes, the results from this assay should be used in combination with clinical examination, patient history, and all other clinical findings.

PRINCIPLE OF THE TEST

Monkey TGFB ELISA kit uses sandwich-ELISA as the method. The microtiter plate provided in this kit has been pre-coated with Anti- TGFB antibody. And the biotin conjugated Anti- TGFB antibody was used as detection antibodies. The standards, test samples and biotin conjugated detection antibody were added to the wells subsequently, and wash with wash buffer. HRP-Streptavidin was added and unbound conjugates were washed away with wash buffer. Then a TMB substrate solution is added to each well. The enzyme-substrate reaction is terminated by the addition of diluted sulphuric acid solution (stop solution) and the color change is measured spectrophotometrically at a wavelength of 450 nm. The concentration of TGFB in the samples is then determined by comparing the OD of the samples to the standard curve.

MATERIALS AND EQUIPMENT REQUIRED

Adjustable micropipette (50-200 μl) and multichannel pipet with disposable plastic tips. Reagent troughs, plate shaker (orbital shaker), plate washer (recommended) and ELISA plates Reader.

SPECIMEN COLLECTION AND STORAGE

Plasma: Collect plasma using EDTA-Na2 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. Avoid hemolysis, high cholesterol samples.
Serum: Allow samples to clot for 2 hours at room temperature or overnight at 4°C before centrifugation for 20 minutes at approximately 1000×g. Collect the supernatant and carry out the assay immediately. Blood collection tubes should be disposable, non-pyrogenic, and non-endotoxin.

Notes:

1. Samples should be used within 7 days when stored at 2-8℃, otherwise samples must be divided and stored at -20℃ (≤1month) or -80℃ (≤6months) to avoid the loss of bioactivity and contamination. Avoid repeated freeze-thaw cycles.
2. Please take the samples to room temperature (18-25℃) without extra heating before performing the assay.
3. 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.

Sample Pre-process
In order to expose the binding site of antigen, we suggest pre-process the sample.

Serum/Plasma: Add 20 ul of Pre-process Agent A to 40 ul samples, mix well and incubate for 10 mins at room temperature. Neutralize the acidified sample by adding 20 ul of Pre-process Agent B, mix well. **Note**: **The matrix components in the sample will affect test results, which need to be diluted at least ½ with sample/ standard dilution buffer before testing.**

Cell Culture Supernatant: Add 20 ul of Pre-process Agent A to 100 ul samples, mix well and incubate for 10 mins at room temperature. Neutralize the acidified sample by adding 20 ul of Pre-process Agent B, mix well. At last, add 60 ul sample/standard dilution buffer, mix well and test. **Note: Sample is diluted 2 times.**

Assay Performance

1. It is important that the time of reaction in each well is held constant to achieve reproducible results.
2. Pipetting of samples should not extend beyond ten (10) minutes to avoid assay drift.
4. If more than one (1) plate is used, it is recommended to repeat the dose response curve.
5. The addition of substrate solution initiates a kinetic reaction, which is terminated by the addition of the stop solution. Therefore, the substrate and stop solution should be added in the same sequence to eliminate any time-deviation during reaction.
6. Plate readers measure vertically. Do not touch the bottom of the wells.
7. Failure to remove adhering solution adequately in the aspiration or decantation wash step(s) may result in poor replication and spurious results.
8. Use components from the same lot. No intermixing of reagents from different batches.
9. Accurate and precise pipetting, as well as following the exact time and temperature requirements prescribed, is essential. Any deviation from ADI's IFU may yield inaccurate results.
10. All applicable national standards, regulations and laws, including, but not limited to, good laboratory procedures, must be strictly followed to ensure compliance and proper device usage.
11. It is important to calibrate all the equipment e.g. Pipettes, Readers, Washers and/or the automated instruments used with this device, and to perform routine preventative maintenance.

REAGENTS PREPARATION FOR THE ASSAY:

Wash Buffer (25X): Dilute the wash buffer with distilled water (dissolve content of 1 bottle (30 ml) into 750 ml deionized or distilled water). Some buffer components may crystallize in wash concentrate. These redissolve at room temperature. Store diluted wash buffer at 2-8ºC.

Standard: 1) 2000 pg/ml of standard solution: Add 1 ml of Sample / Standard dilution buffer into one Standard tube, keep the tube at room temperature for 10 minutes and mix them thoroughly.
2) 1000 pg/ml→31.25 pg/ml of standard solutions: Label 6 Eppendorf tubes with 1000pg/ml, 500pg/ml, 250pg/ml, 125pg/ml, 62.5pg/ml, 31.25pg/ml respectively. Aliquot 0.3 ml of the Sample /Standard dilution buffer into each tube. Add 0.3 ml of the above 2000 pg/ml standard solution into 1st tube and mix thoroughly. Transfer 0.3 ml from 1st tube to 2nd tube and mix thoroughly. Transfer 0.3 ml from 2nd tube to 3rd tube and mix thoroughly, and so on.
Note: The standard solutions are best used within 2 hours. The standard solution should be at 4°C for up to 12 hours. Or store at -20 °C for up to 48 hours. Avoid repeated freeze-thaw cycles.

Biotin- labeled Antibody Working Solution: Prepare it within1 hour before experiment. Dilute the Biotin- labeled Antibody with Antibody Dilution Buffer at **1:100** and mix them thoroughly. (i.e. Add 1 µl of Biotin- labeled Antibody into 99 µl of Antibody Dilution Buffer)

HRP-Streptavidin Conjugate Working Solution: Prepare it within 30 minutes before experiment. Dilute the HRP-Streptavidin Conjugate with HRP-Streptavidin Conjugate dilution buffer at 1:100 and mix thoroughly. (i.e. Add 1 µl of HRP-Streptavidin Conjugate into 99 µl of HRP-Streptavidin Conjugate dilution buffer)

STORAGE AND STABILITY

The microtiter well plate and all other reagents, if unopened, are stable at 2-8ºC until the expiration date printed on the label. The whole kit stability is usually 6 months from the date of shipping under appropriate storage conditions. Do not freeze and thaw.

Quality Control:

Each laboratory should assay controls at levels in the low, normal and high range for monitoring assay performance. These controls should be treated as unknowns and values determined in every test procedure performed. Quality control charts should be maintained to follow the performance of the supplied reagents. Pertinent statistical methods should be employed to ascertain trends. The individual laboratory should set acceptable assay performance limits. In addition, maximum absorbance should be consistent with past experience. Significant deviation from established performance can indicate unnoticed change in experimental conditions or degradation of kit reagents. Fresh reagents should be used to determine the reason for the variations.

TEST PROCEDURE *(ALLOW ALL REAGENTS TO REACH ROOM TEMPERATURE, 25-28oC, BEFORE USE). Prepare working solutions of biotin-antibody, HRP conjugate, Standard and wash buffer (see page 3). Bring all reagents and solutions to room temp. (25-28oC).*

1. Organize the microplates' wells for each standard, control and test sample to be assayed in duplicate. Replace any unused microwell strips back into the aluminum bag, seal and store at 2-8°C.
2. It's recommended to measure each standard and sample in duplicate. Wash plate 2 times with 350 ul wash buffer before adding standard, sample and control (blank) wells.
3. Pipette **100 µl of TGFB standard, Blank or Sample (diluted at least ½ with sample/ standard dilution buffer)** into the assigned well. (The blank well is added with Sample/Standard dilution buffer). Seal the plate with a cover and incubate at 37 °C for 90 min.
4. Remove the cover and discard the plate content. Wash plate 2 times with 350 ul wash buffer. Do NOT let the wells completely dry at any time.
5. Add **100 µl of the Biotin- labeled antibody** to all wells.
6. Mix the microplate gently for 20-30 seconds by gentle tapping against the palm. Cover and **incubate for 60 minutes** at 37°C.
7. Remove the cover and Wash plate 3 times with 350 ul wash buffer. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. An automatic or manual plate washer can be used. Follow the manufacturer's instruction for proper usage. If a squeeze bottle is employed, fill each well by depressing the container (avoiding air bubbles) to dispense the wash. Decant the wash and repeat two (2) additional times.
8. Add **100 µl of HRP-Streptavidin Conjugate** to all wells. Mix the microplate gently for 20-30 seconds by tapping against the palm of your hand..
9. Cover and **incubate for 30 minutes** at 37°C.
10. Wash plate 5 times with 350 ul wash buffer. After the last wash, remove any remaining Wash Buffer by aspirating or decanting.
11. Add **90 µl of TMB substrate** reagent to all wells. Note: Always add reagents in the same order to minimize reaction time differences between wells. DO NOT SHAKE THE PLATE AFTER SUBSTRATE ADDITION. **Incubate at 37°C in dark within 15-30 minutes.**
12. Add **50 µl of stop solution** to each well and gently mix for 15-20 seconds. Always add reagents in the same order to minimize reaction time differences between wells.
13. **Read the absorbance in each well at 450nm**. The results should be read within fifteen (15) minutes of adding the stop solution.