# Quantikine™ ELISA

## Human TGF-β2 Immunoassay

Catalog Number DB250
SB250
PDB250

For the quantitative determination of activated human Transforming Growth Factor beta 2 (TGF-β2) concentrations in cell culture supernates, serum, and plasma.

This package insert must be read in its entirety before using this product.
For research use only. Not for use in diagnostic procedures.

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**Manufactured and Distributed by:**

**USA** R&D Systems, Inc.
614 McKinley Place NE, Minneapolis, MN 55413
**TEL:** 800 343 7475 612 379 2956
**FAX:** 612 656 4400
**E-MAIL:** info@bio-techne.com

**Distributed by:**

**Europe | Middle East | Africa** Bio-Techne Ltd.
19 Barton Lane, Abingdon Science Park
Abingdon OX14 3NB, UK
**TEL:** +44 (0)1235 529449
**FAX:** +44 (0)1235 533420
**E-MAIL:** info.emea@bio-techne.com

**China** Bio-Techne China Co., Ltd.
Unit 1901, Tower 3, Raffles City Changning Office,
1193 Changning Road, Shanghai PRC 200051
**TEL:** +86 (21) 52380373 (400) 821-3475
**FAX:** +86 (21) 52371001
**E-MAIL:** info.cn@bio-techne.com

# INTRODUCTION

TGF-β2 is synthesized as a prepro-cytokine with a 19 amino acid (aa) signal sequence, a 283 aa pro-region, and a 112 aa mature segment (1-5). It dimerizes with formation of disulfide bonds between the 'pro' regions and disulfide bonds between the 'mature' regions. The mature region is 71% and 80% identical with human TGF-β1 and TGF-β3 (6, 7) and 97% identical with the corresponding mouse protein (8). After proteolytic cleavage of the disulfide-linked mature region, it remains hydrogen-bonded to the disulfide-linked prosegments (LAP or latency-associated protein) (1, 2, 9). If secreted in this form, LAP keeps TGF-β2 in an inactive state until dissociation, caused by proteases, glycosidases, or extreme pH (2, 9). In many types of cells, an additional protein, latent TGF-β binding protein (LTBP), is covalently linked to the LAP homodimer prior to secretion. LTBP, a 130 kDa cysteine-rich glycoprotein, creates a 235 kDa large latent complex that is secreted, most likely binding to the extracellular matrix (1, 9-11). The latency components are believed to act as natural antagonists of TGF-β activity, to target TGF-β to distinct tissues, and to maintain a reservoir of TGF-β (1, 2, 12). On release from latency, active homodimeric TGF-β can bind to cell-surface receptors or to other proteins, such as $\alpha_2$-macroglobulin (13).

The signal transducing receptor for TGF-β2 is a heterotetrameric complex of two type I signal-transducing receptors (53 kDa; TGF-β RI) and two type II ligand-binding receptors (75-85 kDa; TGF-β RII) (14-19). The binding of TGF-β2 appears to initially involve a type III TGF-β receptor, either 300 kDa betaglycan (20) or 180 kDa endoglin (14, 21), which then "hands off" to TGF-β RII.

TGF-β2 is expressed by a variety of cells, including osteoclasts, thymic epithelium, keratinocytes, hepatocytes, chief cells of the stomach, satellite cells, skeletal muscle cells, prostatic epithelium, bronchial epithelium, neurons and astrocytes, fibroblasts and visceral smooth muscle, and macrophages (14-19). TGF-β2 has marked cross-species bioactivity (*e.g.*, human TGF-β2 is active on mouse cells (33), while porcine TGF-β2 is active on rabbit cells (34)). TGF-β2 has four fundamental activities: it is a growth inhibitor for most types of cells; it enhances the deposition of extracellular matrix; it is immunosuppressive, suppressing APC expression of both IL-12 and CD40L while upregulating IL-10 secretion; and during fetal development, it is expressed in discrete areas, such as epithelium, myocardium, cartilage and bone of extremities and in the nervous system, suggesting specific functions (1, 35-37).

The Quantikine™ Human TGF-β2 Immunoassay is a 4.5 hour solid-phase ELISA designed to measure activated human TGF-β2 in cell culture supernates, serum, and plasma. It contains NS0-expressed recombinant human TGF-β and has been shown to quantitate the recombinant factor accurately. Results obtained using human TGF-β2 showed linear curves that were parallel to the standard curves obtained using the recombinant TGF-β2 kit standards. These results indicate that this kit can be used to determine relative mass values for natural human TGF-β2.

## PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for human TGF-β2 has been pre-coated onto a microplate. Standards and activated samples are pipetted into the wells and any TGF-β2 present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for human TGF-β2 is added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, a substrate solution is added to the wells and color develops in proportion to the amount of TGF-β2 bound in the initial step. The color development is stopped and the intensity of the color is measured.

## LIMITATIONS OF THE PROCEDURE

- FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
- The kit should not be used beyond the expiration date on the kit label.
- Do not mix or substitute reagents with those from other lots or sources.
- This kit is designed and validated for use with samples activated using the procedure provided.
- If samples generate values higher than the highest standard, further dilute the samples after activation with calibrator diluent and repeat the assay.
- Any variation in diluent, operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.
- Variations in sample collection, processing, and storage may cause sample value differences.
- This assay is designed to eliminate interference by other factors present in biological samples. Until all factors have been tested in the Quantikine™ Immunoassay, the possibility of interference cannot be excluded.

## TECHNICAL HINTS

- When mixing or reconstituting protein solutions, always avoid foaming.
- To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.
- To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
- When using an automated plate washer, adding a 30 second soak period following the addition of Wash Buffer, and/or rotating the plate 180 degrees between wash steps may improve assay precision.
- Substrate Solution should remain colorless until added to the plate. Keep Substrate Solution protected from light. Substrate Solution should change from colorless to gradations of blue.
- Stop Solution should be added to the plate in the same order as the Substrate Solution. The color developed in the wells will turn from blue to yellow upon addition of the Stop Solution. Wells that are green in color indicate that the Stop Solution has not mixed thoroughly with the Substrate Solution.

## MATERIALS PROVIDED & STORAGE CONDITIONS

Store the unopened kit at 2-8 °C. Do not use past kit expiration date.

| PART | PART # | CATALOG # DB250 | CATALOG # SB250 | DESCRIPTION | STORAGE OF OPENED/ RECONSTITUTED MATERIAL |
|---|---|---|---|---|---|
| **Human TGF-β2 Microplate** | 890437 | 1 plate | 6 plates | 96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody specific for human TGF-β2. | Return unused wells to the foil pouch containing the desiccant pack. Reseal along entire edge of the zip-seal. May be stored for up to 1 month at 2-8 °C.* |
| **Human TGF-β2 Standard** | 890267 | 2 vials | 12 vials | Recombinant human TGF-β2 in a buffered protein base with preservatives; lyophilized. *Refer to the vial label for reconstitution volume.* | Discard after use. Use a fresh standard for each assay. |
| **Human TGF-β2 Conjugate** | 890438 | 1 vial | 6 vials | 21 mL/vial of polyclonal antibody specific for human TGF-β2 conjugated to horseradish peroxidase with preservatives. | May be stored for up to 1 month at 2-8 °C.* |
| **Assay Diluent RD1-17** | 895433 | 1 vial | 6 vials | 11 mL/vial of a buffered protein base with preservatives. | |
| **Calibrator Diluent RD5I** | 895134 | 3 vials | 18 vials | 21 mL/vial of a buffered protein base with preservatives. | |
| **Wash Buffer Concentrate** | 895003 | 1 vial | 6 vials | 21 mL/vial of a 25-fold concentrated solution of buffered surfactant with preservative. *May turn yellow over time.* | |
| **Color Reagent A** | 895000 | 1 vial | 6 vials | 12 mL/vial of stabilized hydrogen peroxide. | |
| **Color Reagent B** | 895001 | 1 vial | 6 vials | 12 mL/vial of stabilized chromogen (tetramethylbenzidine). | |
| **Stop Solution** | 895032 | 1 vial | 6 vials | 6 mL/vial of 2N sulfuric acid. | |
| **Plate Sealers** | N/A | 4 strips | 24 strips | Adhesive strips. | |

* Provided this is within the expiration date of the kit.

DB250 contains sufficient materials to run an ELISA on one 96 well plate.
SB250 (SixPak) contains sufficient materials to run ELISAs on six 96 well plates.

This kit is also available in a PharmPak (R&D Systems®, Catalog # PDB250). Refer to the PharmPak Contents section for specific vial counts.

## PHARMPAK CONTENTS

Each PharmPak contains reagents sufficient for the assay of 50 microplates (96 wells/plate). The package inserts supplied are the same as those supplied in the single kit packs and because of this, a few minor differences related to the number of reagents and their container sizes should be noted.

- Sufficient material is supplied to perform at least 50 standard curves; reuse of each vial may be required. The number of vials, and the number of standard curves obtained per vial will vary with the analyte.
- Wash Buffer 25X Concentrate is bulk packed in 125 mL bottles containing 100 mL.
  **Note:** *Additional wash buffer is available for purchase (R&D Systems®, Catalog # WA126).*

The reagents provided in this PharmPak are detailed below.

| PART | PART # | QUANTITY |
|---|---|---|
| Human TGF-β2 Microplate | 890437 | 50 plates |
| Human TGF-β2 Standard | 890267 | 50 vials |
| Human TGF-β2 Conjugate | 890438 | 50 vials |
| Assay Diluent RD1-17 | 895433 | 50 vials |
| Calibrator Diluent RD5I | 895134 | 150 vials |
| Wash Buffer Concentrate | 895126 | 9 bottles |
| Color Reagent A | 895000 | 50 vials |
| Color Reagent B | 895001 | 50 vials |
| Stop Solution | 895032 | 50 vials |
| Plate Sealers | N/A | 100 sheets |
| Package Inserts | 750178 | 2 booklets |

**If additional standard vials are needed, contact Technical Service at techsupport@bio-techne.com*

## ADDITIONAL REAGENTS REQUIRED

- HEPES, free acid (ACS Grade, M.W. 238.3)
- Hydrochloric acid (ACS Grade, 12 N)
- Sodium hydroxide (ACS Grade, 10 N)

## OTHER SUPPLIES REQUIRED

- Microplate reader capable of measuring absorbance at 450 nm, with the correction wavelength set at 540 nm or 570 nm
- Pipettes and pipette tips
- Deionized or distilled water
- Squirt bottle, manifold dispenser, or automated microplate washer
- 500 mL graduated cylinder
- **Polypropylene** test tubes for dilution of standards and samples
- Human TGF-β2 Controls (optional; R&D Systems®, Catalog # QC20)

## PRECAUTIONS

The Stop Solution provided with this kit is an acid solution.

Some components in this kit contain a preservative which may cause an allergic skin reaction. Avoid breathing mist.

Color Reagent B may cause skin, eye, and respiratory irritation. Avoid breathing fumes.

Wear protective gloves, clothing, eye, and face protection. Wash hands thoroughly after handling. Refer to the SDS on our website prior to use.

## SAMPLE COLLECTION & STORAGE

**The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.**

**Cell Culture Supernates** - Remove particulates by centrifugation and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

**Note:** *A 10% solution of bovine serum used in cell culture can be expected to contribute typically 100-200 pg/mL of bTGF-β2 upon activation. Prepare, activate and assay an appropriate media control to determine the baseline bTGF-β2 in the sample.*

**Serum** - Use a serum separator tube (SST) and allow samples to clot for 30 minutes at room temperature before centrifugation for 15 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

**Plasma** - Collect plasma using EDTA, heparin, or citrate as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

**Note:** *Grossly hemolyzed samples are not suitable for use in this assay.*

## ACTIVATION REAGENT PREPARATION

To activate latent TGF-β2 to the immunoreactive form, prepare the following solutions for acid activation and neutralization. The solutions may be stored in polypropylene bottles at room temperature for up to one month.

**Caution:** *Wear protective clothing and safety glasses during preparation and use of these reagents.*

**1 N HCl (100 mL)** - To 91.67 mL of deionized water, slowly add 8.33 mL of 12 N HCl. Mix well.

**1.2 N NaOH/0.5 M HEPES (100 mL)** - To 75 mL of deionized water, slowly add 12 mL of 10 N NaOH. Mix well. Add 11.9 g of HEPES. Mix well. Bring final volume to 100 mL with deionized water.

## ACTIVATION PROCEDURE

**Use polypropylene tubes.** Do not activate the kit standards as they already contain active TGF-β2.

1. To 125 µL of sample add 25 µL of 1 N HCl. Mix well.
2. Incubate 10 minutes at room temperature.
3. Add 25 µL of 1.2 N NaOH/0.5 M HEPES. Mix well.
4. Add 800 µL of Calibrator Diluent RD5I. Mix well and assay within 2 hours.

**Note:** *Sample results must be multiplied by the dilution factor, 7.8. If samples generate values higher than the highest standard, further dilute the samples after activation with the calibrator diluent and repeat the assay.*

## REAGENT PREPARATION

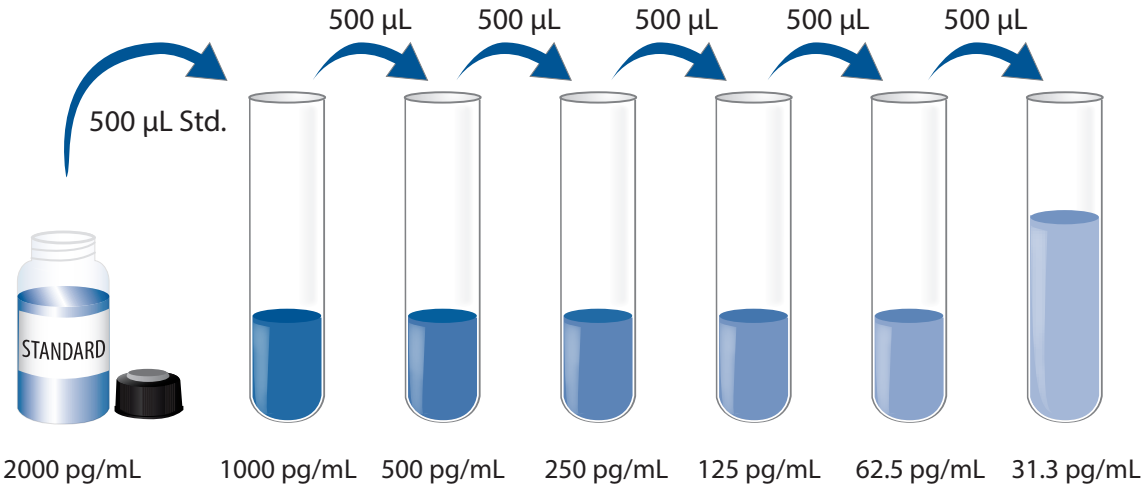
**Bring all reagents to room temperature before use.**

**Wash Buffer** - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Add 20 mL of Wash Buffer Concentrate to 480 mL of deionized or distilled water to prepare 500 mL of Wash Buffer.

**Substrate Solution** - Color Reagents A and B should be mixed together in equal volumes within 15 minutes of use. Protect from light. 200 µL of the resultant mixture is required per well.

**Human TGF-β2 Standard** - **Refer to the vial label for reconstitution volume.** Reconstitute the Human TGF-β2 Standard with Calibrator Diluent RD5I. This reconstitution produces a stock solution of 2000 pg/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions.

**Use polypropylene tubes.** Pipette 500 µL of Calibrator Diluent RD5I into each tube. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The undiluted standard serves as the high standard (2000 pg/mL). Calibrator Diluent RD5I serves as the zero standard (0 pg/mL).



## ASSAY PROCEDURE

**Bring all reagents and samples to room temperature before use. It is recommended that all standards, controls, and samples be assayed in duplicate.**

1. Prepare all reagents, working standards, and samples* as directed in the previous sections.
2. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal.
3. Add 100 µL of Assay Diluent RD1-17 to each well.
4. Add 100 µL of standard, control, or activated sample* per well. Cover with the adhesive strip provided. Incubate for 2 hours at room temperature. A plate layout is provided as a record of samples and standards assayed.
5. Aspirate each well and wash, repeating the process twice for a total of three washes. Wash by filling each well with Wash Buffer (400 µL) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
6. Add 200 µL of Human TGF-β2 Conjugate to each well. Cover with a new adhesive strip. Incubate for 2 hours at room temperature.
7. Repeat the aspiration/wash as in step 5.
8. Add 200 µL of Substrate Solution to each well. Incubate for 20 minutes at room temperature. **Protect from light.**
9. Add 50 µL of Stop Solution to each well. The color in the wells should change from blue to yellow. If the color in the wells is green or the color change does not appear uniform, gently tap the plate to ensure thorough mixing.
10. Determine the optical density of each well within 30 minutes, using a microplate reader set to 450 nm. If wavelength correction is available, set to 540 nm or 570 nm. If wavelength correction is not available, subtract readings at 540 nm or 570 nm from the readings at 450 nm. This subtraction will correct for optical imperfections in the plate. Readings made directly at 450 nm without correction may be higher and less accurate.

*Samples require activation. Refer to page 6 for protocol.

## CALCULATION OF RESULTS

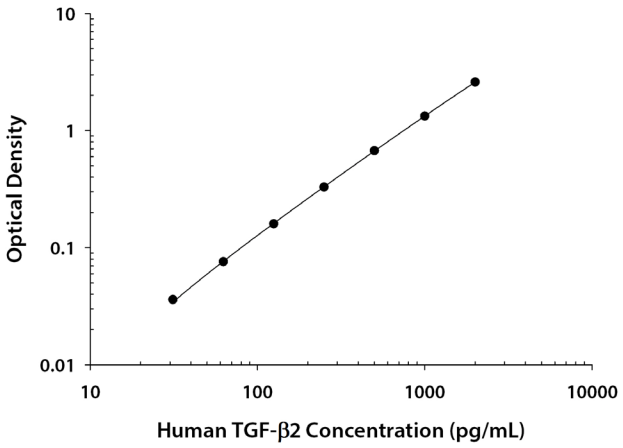
Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density (O.D.).

Create a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the log of the human TGF-β2 concentrations versus the log of the O.D. and the best fit line can be determined by regression analysis. This procedure will produce an adequate but less precise fit of the data.

Since samples have been diluted in the activation step, the concentration read from the standard curve must be multiplied by the dilution factor, 7.8.

## TYPICAL DATA

This standard curve is provided for demonstration only. A standard curve should be generated for each set of samples assayed.




| (pg/mL) | O.D. | Average | Corrected |
|---|---|---|---|
| 0 | 0.031<br>0.033 | 0.032 | — |
| 31.3 | 0.067<br>0.068 | 0.068 | 0.036 |
| 62.5 | 0.106<br>0.109 | 0.108 | 0.076 |
| 125 | 0.187<br>0.197 | 0.192 | 0.160 |
| 250 | 0.357<br>0.364 | 0.361 | 0.329 |
| 500 | 0.701<br>0.709 | 0.705 | 0.673 |
| 1000 | 1.316<br>1.406 | 1.361 | 1.329 |
| 2000 | 2.607<br>2.638 | 2.623 | 2.591 |

## PRECISION

**Intra-Assay Precision** (Precision within an assay)
Three samples of known concentration were tested twenty times on one plate to assess intra-assay precision.

**Inter-Assay Precision** (Precision between assays)
Three samples of known concentration were tested in twenty separate assays to assess inter-assay precision.

| | Intra-Assay Precision | | | Inter-Assay Precision | | |
|---|---|---|---|---|---|---|
| **Sample** | **1** | **2** | **3** | **1** | **2** | **3** |
| **n** | 20 | 20 | 20 | 20 | 20 | 20 |
| **Mean (pg/mL)** | 194 | 509 | 1044 | 196 | 500 | 1015 |
| **Standard deviation** | 13.8 | 27.0 | 28.3 | 9.8 | 21.5 | 43.6 |
| **CV (%)** | 7.1 | 5.3 | 2.7 | 5.0 | 4.3 | 4.3 |

## RECOVERY

To assess recovery of the assay, samples were spiked with three concentrations of human TGF-β2, activated, and measured in the assay.

| Sample Type | Average % Recovery | Range |
|---|---|---|
| Cell culture media (n=5) | 101 | 89-113% |
| Serum (n=5) | 86 | 73-95% |
| EDTA plasma (n=5) | 101 | 95-111% |
| Heparin plasma (n=5) | 102 | 89-113% |
| Citrate plasma (n=5) | 107 | 93-116% |

## LINEARITY

To assess the linearity of the assay, samples were spiked with high concentrations of human TGF-β2, activated and then serially diluted with calibrator diluent to produce samples with values within the dynamic range of the assay.

| | | Cell culture media (n=5) | Serum (n=5) | EDTA plasma (n=5) | Heparin plasma (n=5) | Citrate plasma (n=5) |
|---|---|---|---|---|---|---|
| 1:2 | Average % of Expected | 97 | 99 | 101 | 100 | 95 |
| | Range (%) | 96-98 | 96-100 | 97-107 | 97-102 | 85-104 |
| 1:4 | Average % of Expected | 90 | 93 | 94 | 92 | 87 |
| | Range (%) | 88-91 | 89-101 | 90-103 | 86-101 | 79-95 |
| 1:8 | Average % of Expected | 93 | 95 | 97 | 97 | 90 |
| | Range (%) | 91-95 | 92-100 | 92-107 | 90-107 | 81-97 |
| 1:16 | Average % of Expected | 94 | 99 | 100 | 100 | 93 |
| | Range (%) | 92-95 | 95-106 | 95-108 | 93-109 | 82-99 |

## SENSITIVITY

The minimum detectable dose (MDD) of human TGF-β2 is typically less than 7.0 pg/mL.

The MDD was determined by adding two standard deviations to the mean O.D. value of twenty zero standard replicates and calculating the corresponding concentration.

## CALIBRATION

This immunoassay is calibrated against a highly purified NS0-expressed recombinant human TGF-β2 produced at R&D Systems®.

The NIBSC Non-WHO reference materials 89/518 (natural bovine) and 90/696 (recombinant human) were evaluated in this kit. The dose response of both preparations parallels the Quantikine™ standard curve. Use the equations below to convert sample values obtained with the Quantikine Human TGF-β2 kit to approximate NIBSC units.

NIBSC (89/518) approximate value (U/mL) = 0.0551 x Quantikine TGF-β2 value (pg/mL)

NIBSC (90/696) approximate value (U/mL) = 0.0272 x Quantikine TGF-β2 value (pg/mL)

## SAMPLE VALUES

**Serum/Plasma** - Samples from apparently healthy volunteers were evaluated for the presence of human TGF-β2 in this assay. No medical histories were available for the donors used in this study.

| Sample Type | Mean of Detectable (pg/mL) | % Detectable | Range (pg/mL) |
|---|---|---|---|
| Serum (n=63) | 386 | 86 | ND-873 |
| EDTA plasma (n=63) | 302 | 49 | ND-442 |
| Citrate plasma (n=63) | 278 | 21 | ND-320 |
| Heparin plasma (n=63) | 325 | 54 | ND-454 |

ND=Non-detectable

**Cell Culture Supernates:**

Human PBMCs did not produce detectable levels of human TGF-β2 upon activation.

MCF-7 human breast cancer cells were cultured unstimulated and stimulated with PMA for 3 days. The culture media and supernate were activated and evaluated for the presence of human TGF-β2 in this assay.

| Condition | Day 3 (pg/mL) |
|---|---|
| Media | 143 |
| Unstimulated MCF-7 supernate | 376 |
| Stimulated MCF-7 supernate | 1038 |

## SPECIFICITY

This assay recognizes natural and recombinant human TGF-β2. This assay also recognizes natural porcine TGF-β2.

The factors listed below were prepared at 50 ng/mL in calibrator diluent, activated, and assayed for cross-reactivity. Preparations of the following factors at 50 ng/mL in a mid-range human TGF-β2 control were activated and assayed for interference. No significant cross-reactivity or interference was observed.

**Recombinant human:**
TGF-α
TGF-β1
TGF-β RII
TGF-β RIII

**Other recombinants:**
amphibian TGF-β5
chicken TGF-β3

**Natural proteins:**
porcine TGF-β1

Porcine TGF-β1.2 cross-reacts approximately 0.48% in this assay.

The following cytokines were found to interfere only in cell culture supernate samples and did not interfere in serum samples.

| Recombinant Factor | Amount of Cytokine Added (ng/mL) | TGF-β2 Result (pg/mL) |
|---|---|---|
| Human LAP (TGF-β1) | 0 | 5327 |
| | 30 | 4726 |
| | 50 | 4500 |
| Human Latent TGF-β1 | 0 | 5327 |
| | 30 | 4709 |
| | 40 | 4381 |

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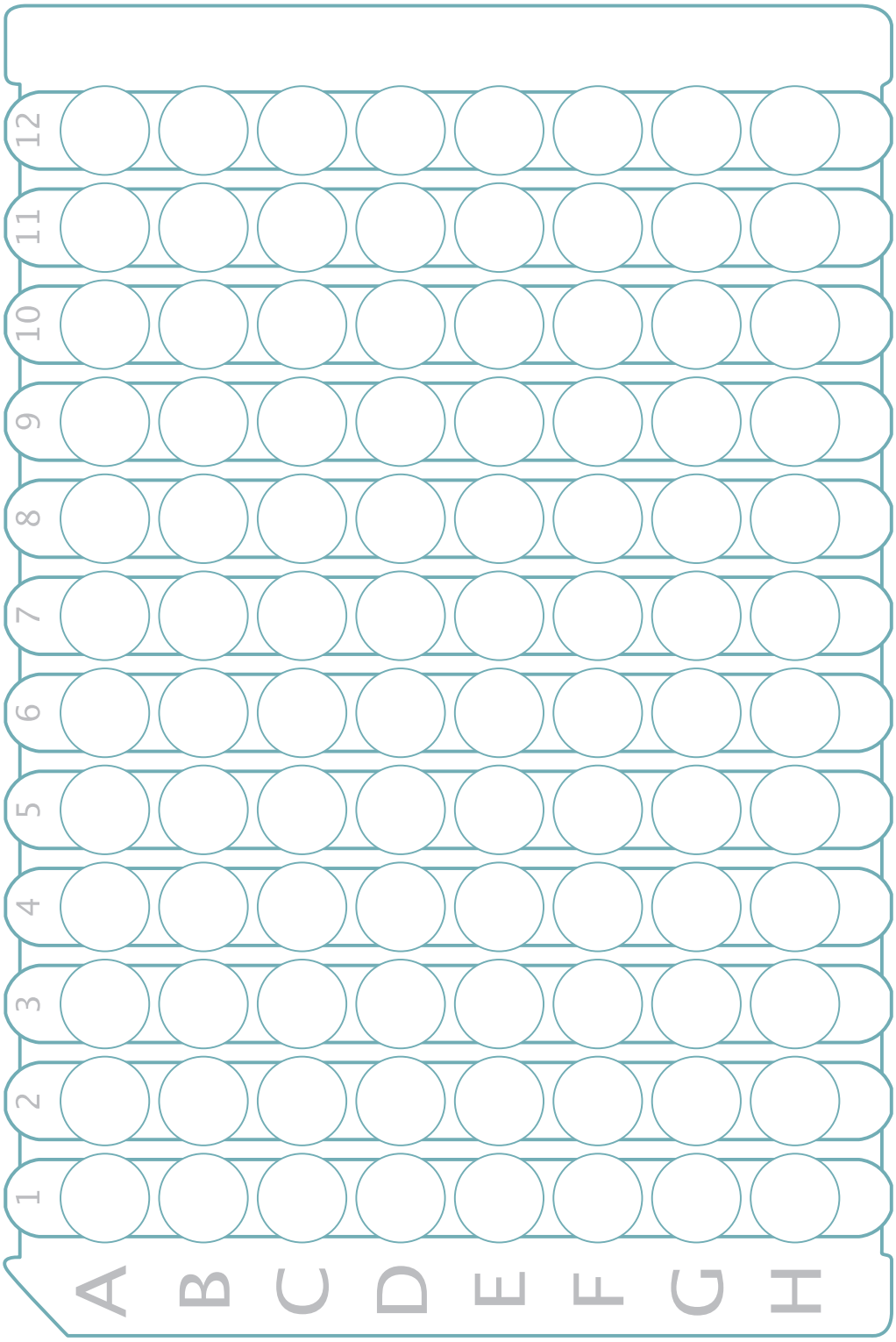
## PLATE LAYOUT

Use this plate layout to record standards and samples assayed.

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