



**Promega**

## Technical Bulletin

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# TGF $\beta$ <sub>1</sub> E<sub>max</sub><sup>®</sup> ImmunoAssay System

INSTRUCTIONS FOR USE OF PRODUCTS G7590 AND G7591.



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# TGF $\beta$ <sub>1</sub> E<sub>max</sub><sup>®</sup> ImmunoAssay System

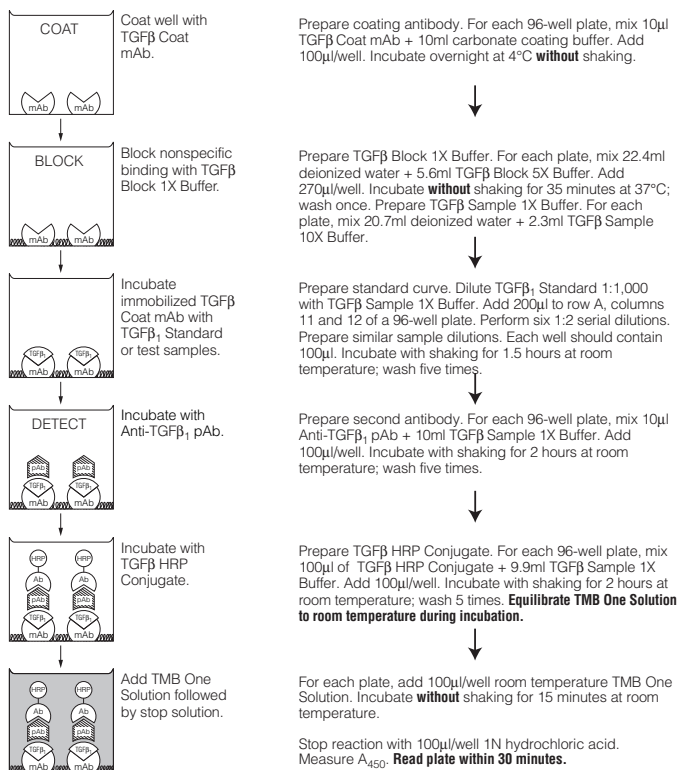
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## 1. Description

The TGF $\beta$ <sub>1</sub> E<sub>max</sub><sup>®</sup> ImmunoAssay System is designed for the sensitive and specific detection of biologically active TGF $\beta$ <sub>1</sub> in an antibody sandwich format (1) (Figure 1). In this format, flat-bottom 96-well plates are coated with TGF $\beta$  Coat mAb, which binds soluble TGF $\beta$ <sub>1</sub>. The captured TGF $\beta$ <sub>1</sub> is bound by a second specific polyclonal antibody (pAb). After washing, the amount of specifically bound pAb is detected using a species-specific antibody conjugated to horseradish peroxidase (TGF $\beta$  HRP Conjugate) as a tertiary reactant. The unbound conjugate is removed by washing, and following an incubation with a chromogenic substrate, the color change is measured. The amount of TGF $\beta$ <sub>1</sub> in

the test solutions is proportional to the color generated in the oxidation-reduction reaction. Using this system, biologically active TGF $\beta$ <sub>1</sub> in tissue culture supernatants, plasma, serum or urine can be quantitated in the range of 32-1,000pg/ml.



**Figure 1. Schematic representation of the TGF $\beta$ <sub>1</sub> E<sub>max</sub>® ImmunoAssay System.** For a detailed protocol, or when using this system for the first time, please read Sections 3 through 4 carefully.

The TGF $\beta$ <sub>1</sub> E<sub>max</sub>® ImmunoAssay System offers several benefits:

- **Specificity:** Specific detection of TGF $\beta$ <sub>1</sub>; typically less than or equal to 3% cross-reactivity with TGF $\beta$ <sub>2</sub> and TGF $\beta$ <sub>3</sub> at 10ng/ml.
- **Sensitivity:** Detects a minimum of 32pg/ml of TGF $\beta$ <sub>1</sub>.
- **Flexibility:** ELISA plates can be set up in any desired configuration.
- **High Value:** Optimized reagents and protocol.

## 2. Product Components and Storage Conditions

The TGFβ<sub>1</sub> E<sub>max</sub><sup>®</sup> ImmunoAssay System is offered in two sizes, which differ only in the quantity of reagents, sufficient for either five plates or two plates. Catalog numbers and quantity of reagents are listed below.

Product	Size	Cat.#
TGFβ <sub>1</sub> E <sub>max</sub> <sup>®</sup> ImmunoAssay System	2 × 96 wells	G7590

Each system contains sufficient reagents for 160 sample determinations plus standards (plates not included). Includes:

- 20µl TGFβ Coat mAb
- 12ml TGFβ Block 5X Buffer
- 8ml TGFβ Sample 10X Buffer
- 20µl TGFβ<sub>1</sub> Standard, 1µg/ml
- 20µl Anti-TGFβ<sub>1</sub> pAb, TGFβ<sub>1</sub> ELISA
- 200µl TGFβ HRP Conjugate
- 25ml TMB One Solution

Product	Size	Cat.#
TGFβ <sub>1</sub> E <sub>max</sub> <sup>®</sup> ImmunoAssay System	5 × 96 wells	G7591

Each system contains sufficient reagents for 400 sample determinations plus standards (plates not included). Includes:

- 50µl TGFβ Coat mAb
- 28ml TGFβ Block 5X Buffer
- 20ml TGFβ Sample 10X Buffer
- 45µl TGFβ<sub>1</sub> Standard, 1µg/ml
- 50µl Anti-TGFβ<sub>1</sub> pAb, TGFβ<sub>1</sub> ELISA
- 500µl TGFβ HRP Conjugate
- 2 × 25ml TMB One Solution

**Storage Conditions:** Store the entire system in its original package protected from light at -20°C, where it is stable for six months from the date of purchase. Once thawed, store at 4°C, where the system is stable for three months. Return each component to 4°C immediately after use. Avoid refreezing reagents. Use reagents the same day as dilution. Do not add any preservatives to these diluted solutions, as they may interfere with the assay.

**Note:** The TGFβ<sub>1</sub> Standard supplied with this system is recombinant human TGFβ<sub>1</sub>.

### 3. General Considerations

The TGFβ<sub>1</sub> E<sub>max</sub><sup>®</sup> ImmunoAssay System has been tested using the following protocols. Plate coating requires an overnight incubation at 4°C. The subsequent steps require approximately 7 hours to complete and should be performed on the following day.

#### Limitations of the Assay

- For research use only. Not for use in diagnostic procedures.
- Absorbance values beyond the range of the standard curve are not valid.
- For consistent results, dilute samples using the TGFβ Sample 1X Buffer.
- Citrated plasma samples will interfere with the assay. Use EDTA as an anticoagulant to avoid platelet degranulation.

### 4. Sample Preparation

The TGFβ<sub>1</sub> E<sub>max</sub><sup>®</sup> ImmunoAssay System may be used to quantitate TGFβ<sub>1</sub> in tissue culture supernatants, plasma, serum or urine. For plasma samples, use EDTA as an anticoagulant to avoid platelet degranulation. Store experimental samples frozen at -20°C before use. Avoid multiple freeze-thaw cycles. Remove particulates from samples by centrifugation before use in the assay.

Due to the high levels of endogenous TGFβ<sub>1</sub> and binding proteins in serum and plasma, we recommend a starting dilution of:

1:16 (in TGFβ Sample 1X Buffer) for samples containing 10% fetal bovine serum (e.g., conditioned medium), or

1:150 (in TGFβ Sample 1X Buffer) for human serum and plasma samples.

When serially diluting the TGFβ<sub>1</sub> Standard and experimental samples within the plate, take care not to touch or scratch the surfaces of the wells with the pipette tips. If you are unfamiliar with this technique, you may wish to perfect this on a "practice plate."

In vivo, TGFβ<sub>1</sub> is processed from a latent form to the bioactive form of the protein. Only the bioactive form is immunoreactive and detectable by TGFβ<sub>1</sub> monoclonal and polyclonal antibody. This processing can be performed in vitro by acid treatment.

**Note:** This assay is designed to measure biologically active TGFβ<sub>1</sub>. To measure the amount of naturally processed TGFβ<sub>1</sub> in your samples, proceed directly to the ELISA protocol in Section 5.A without acid treatment. To assay for total TGFβ<sub>1</sub>, perform the acid treatment procedure and then neutralize the samples as described before proceeding with the ELISA protocol.

The TGF $\beta$ <sub>1</sub> Standard supplied with the system has been acid treated and will be neutralized upon dilution in TGF $\beta$  Sample 1X Buffer. **Do not attempt to acid treat the TGF $\beta$ <sub>1</sub> Standard.**

### Acid Treatment Procedure

This procedure acidifies samples diluted 1:5 in Dulbecco's PBS (DPBS) to approximately pH 2.6 and then neutralizes them to approximately pH 7.6. Depending on how much carrier protein the samples contain, additional dilutions may or may not require the use of TGF $\beta$  Sample 1X Buffer (which contains a proprietary carrier protein) to minimize loss of TGF $\beta$ <sub>1</sub>.

For urine and other low protein matrices, we recommend direct acid treatment to a pH of 2.0–3.0 for 15–20 minutes. Following neutralization with NaOH, subsequent dilutions, if necessary, should be done with TGF $\beta$  Sample 1X Buffer before adding samples to your assay plate.

For all matrices, verify that the pH is 3.0 or lower. In animal sera, the amount of 1N HCl required to lower the pH will vary depending upon the species. We suggest adding 110–125 $\mu$ l of 1N HCl per milliliter of undiluted serum or plasma and checking the pH before adding additional amounts of acid. Samples can be acid treated in advance and stored at –20°C or at –70°C.

### Materials to Be Supplied by the User

(Solution compositions are provided in Section 8.B.)

- DPBS
- 1N HCl, reagent grade
- 1N NaOH, reagent grade

**Caution:** HCl and NaOH are caustic. Avoid contact with skin or eyes.

1. Dilute the sample by adding 4 volumes of DPBS.
2. Add 1 $\mu$ l of 1N HCl for each 50 $\mu$ l of diluted sample. Verify that the pH is 3.0 or lower.
3. Mix and incubate for 15 minutes at room temperature.
4. Neutralize by adding 1 $\mu$ l of 1N NaOH per 50 $\mu$ l of sample. Check the pH to ensure that it is approximately 7.6.

## 5. Protocol for TGF $\beta$ <sub>1</sub> Quantitation

### Materials to Be Supplied by the User

(Solution compositions are provided in Section 8.B.)

- 96-well (flat-bottom) ELISA plate
- carbonate coating buffer
- plate sealer
- TBST wash buffer
- 1N hydrochloric acid
- microplate reader capable of monitoring absorbance at 450nm
- pipettors capable of accurately delivering volumes of 1 $\mu$ l-1ml
- multichannel pipettor
- wash bottle or automated plate washer (DYNEX UltraWash Plus or equivalent)
- plate shaker (DYNEX Micro-Shaker® or equivalent)
- 50ml (for better mixing) or 15ml polypropylene tubes for dilutions
- 37°C incubator

**Note:** This assay has been tested using Nunc MaxiSorp™ plates (Nunc Cat.# 439454) and Microtiter®-Immunoassay Microplates (Immulon 4; Thermo Labsystems Cat.# 3855). There are no observable differences in the performances of plates from these manufacturers. For best well-to-well accuracy, we recommend a high-quality, name-brand polystyrene ELISA plate.

### 5.A. Plate Coating

1. In a 15ml or 50ml polypropylene tube, add exactly 10 $\mu$ l of the TGF $\beta$  Coat mAb to 10ml of carbonate coating buffer to prepare enough reagent for each full 96-well plate. **Mix thoroughly**, but avoid creating excess bubbles. Use a multichannel pipettor to add 100 $\mu$ l to each well of a polystyrene ELISA plate.



**Note:** The carbonate coating buffer should not be stored for longer than one week, as the pH is unstable over longer periods of time.

**Hint:** Keep the undiluted TGF $\beta$  coat mAb on ice when removed from 4°C storage.

2. Seal the wells with a plate sealer and incubate overnight at 4°C.

**Note:** This assay has been optimized using the carbonate coating buffer prepared as described in Section 8.B; other buffers may give poor results.

## 5.B. Blocking the Plate

Each 96-well plate requires a total of 28ml of TGF $\beta$  Block 1X Buffer.

**Do Not** allow the wells to dry out completely between steps.

1. For each 96-well plate, add 22.4ml of deionized water into a clean 50ml polypropylene tube. Remove 5.6ml of the TGF $\beta$  Block 5X Buffer with a sterile pipette, being careful not to contaminate the stock solution, and add it to the tube. Mix gently and completely by inversion prior to use.
2. Remove the coated plate from the refrigerator and allow it to warm to room temperature (approximately 10–15 minutes). Over a sink, flick out the contents of the wells and slap the plate upside down three times on a paper towel to help clear the wells. Add 270 $\mu$ l of TGF $\beta$  Block 1X Buffer to each well using a multichannel pipettor. Do not touch or scratch the surfaces of the wells where antibody has bound to the plate.
3. Incubate at 37°C for 35 minutes without shaking. **Do not** stack the plates when incubating at 37°C.
4. Wash once as described in Section 5.C.

## 5.C. Washing Procedure

Using the recommended TBST wash buffer (see Section 8.B), wash all wells vigorously using an automated plate washer. Alternatively, plates can be washed by entirely filling each well with wash buffer, flicking out the contents over a sink and slapping the plate three times on a paper towel. Immediately proceed to the next step.

**Note:** We strongly recommend using an automated plate washer for more consistent results.

## 5.D. Preparing the Sample Buffer

Each 96-well plate requires approximately 23ml of TGF $\beta$  Sample 1X Buffer. This volume includes 3ml to prepare the TGF $\beta$ <sub>1</sub> standard curve.

For each 96-well plate, add 20.7ml of deionized water into a clean 50ml polypropylene tube. Remove 2.3ml of the TGF $\beta$  Sample 10X Buffer with a sterile pipette, being careful not to contaminate the stock solution, and add it to the tube. Mix gently and completely by inversion prior to use.

### 5.E. Preparing the TGFβ<sub>1</sub> Standard Curve

The TGFβ<sub>1</sub> standard curve is linear between 15.6 and 1,000pg/ml of the TGFβ<sub>1</sub> Standard. The TGFβ<sub>1</sub> Standard is supplied at a concentration of 1μg/ml and is already acid treated. Accurately dilute the supplied TGFβ<sub>1</sub> Standard 1:1,000 in TGFβ Sample 1X Buffer to achieve a concentration of 1,000pg/ml. For example, dilute 5μl of the TGFβ<sub>1</sub> Standard into 245μl of TGFβ Sample 1X Buffer (1:50 dilution), then dilute 25μl of this into 475μl of the TGFβ Sample 1X Buffer for a final dilution of 1:1,000.

**Hint:** Keep the undiluted TGFβ<sub>1</sub> Standard on ice when removed from 4°C storage.

1. Following plate blocking and washing, designate two columns of wells (16 wells) for the standard curve. To prepare the TGFβ<sub>1</sub> standard curve within the assay plate, add 100μl/well of the sample diluent (Sample 1X Buffer) to wells B through H in the two columns designated for the standard curve (Figure 2).
2. Add 200μl of the diluted TGFβ<sub>1</sub> Standard (1,000pg/ml) to the first well in each column designated for the standard curve (row A).
3. Immediately perform serial 1:2 dilutions (100μl/well) down the plate in the columns designated for the standard curve. In the last set of wells for the standard curve, do not add any TGFβ<sub>1</sub>. The final concentrations (in duplicate) within the plate will be 0–1,000pg/ml (Figure 2).

	Test Samples										TGFβ <sub>1</sub> Standard Curve		
	1	2	3	4	5	6	7	8	9	10	11	12	pg/ml
A	○	○	○	○	○	○	○	○	○	○	○	○	1000
B	○	○	○	○	○	○	○	○	○	○	○	○	500
C	○	○	○	○	○	○	○	○	○	○	○	○	250
D	○	○	○	○	○	○	○	○	○	○	○	○	125
E	○	○	○	○	○	○	○	○	○	○	○	○	62
F	○	○	○	○	○	○	○	○	○	○	○	○	31
G	○	○	○	○	○	○	○	○	○	○	○	○	15.6
H	○	○	○	○	○	○	○	○	○	○	○	○	0

06281A05\_4A

**Figure 2. Recommended ELISA plate format for standard curve and test samples.**

## 5.F. Addition of Sample

We recommend starting with a 1:4 dilution of each test sample and preparing 1:2 serial dilutions per column in the ELISA plate. Alternatively, you may wish to screen samples at a single concentration (perhaps in triplicate) and subsequently reassay all positive samples to determine the exact TGF $\beta$ <sub>1</sub> concentration. Please see the recommended starting dilutions for serum, plasma or culture media in Section 4.

**Note:** Serial dilutions of a sample may not be linear. Use the dilution for your sample that gives absorbance near the middle of your standard curve.

Where the sample carrier solution may contribute nonspecific sources of TGF $\beta$ <sub>1</sub> (such as serum in culture media), we also recommend performing a series of negative control reactions containing the carrier solution alone.

1. After preparing the TGF $\beta$ <sub>1</sub> standard curve, add 100 $\mu$ l of the acid-treated (or naturally processed) samples to each of the remaining wells. (See Section 4 for acid treatment.)



**Note:** Add samples as quickly as possible to minimize evaporation.

2. Incubate the plate for 90 minutes at room temperature with shaking (500  $\pm$  100rpm).

**Note:** Best results are obtained using a plate shaker. Alternatively, plates may be incubated at 37°C without shaking, although a slight loss in sensitivity of the assay may occur.

3. Wash five times as described in Section 5.C.

## 5.G. Addition of Anti-TGF $\beta$ <sub>1</sub> pAb

1. In a 15ml or 50ml polypropylene tube, add 10 $\mu$ l of the Anti-TGF $\beta$ <sub>1</sub> pAb to 10ml of TGF $\beta$  Sample 1X Buffer (1:1,000 dilution) to prepare enough reagent for a full 96-well plate. Mix thoroughly, but avoid creating excess bubbles. Use a multichannel pipettor to add 100 $\mu$ l of the diluted Anti-TGF $\beta$ <sub>1</sub> pAb to each well, being careful not to touch or scratch the surfaces of the wells.

**Hint:** Keep the undiluted Anti-TGF $\beta$ <sub>1</sub> on ice when removed from 4°C storage.

2. Incubate for 2 hours at room temperature with shaking (500  $\pm$  100rpm).

**Note:** Best results are obtained using a plate shaker. Alternatively, plates may be incubated without shaking, although a slight loss in sensitivity of the assay may occur.

3. Wash five times as described in Section 5.C.

### 5.H. Addition of TGF $\beta$ HRP Conjugate

1. In a 15ml or 50ml polypropylene tube, accurately add 100 $\mu$ l of the stock TGF $\beta$  HRP Conjugate to 9.9ml of TGF $\beta$  Sample 1X Buffer (1:100 dilution) to prepare enough reagent for a full 96-well plate. Mix thoroughly, but avoid creating excess bubbles. Using a multichannel pipettor, add 100 $\mu$ l of the diluted TGF $\beta$  HRP Conjugate to each well, being careful not to touch or scratch the surfaces of the wells.

**Hint:** During the incubation with the HRP conjugate, equilibrate the TMB One Solution to room temperature.

2. Incubate for 2 hours at room temperature with shaking (500  $\pm$  100rpm).

**Note:** Best results are obtained using a plate shaker. Alternatively, plates may be incubated without shaking, although a slight loss in sensitivity of the assay may occur.

3. Wash five times as described in Section 5.C.

### 5.I. Color Development

1. Add 100 $\mu$ l of the room temperature TMB One Solution to each well using a multichannel pipettor.



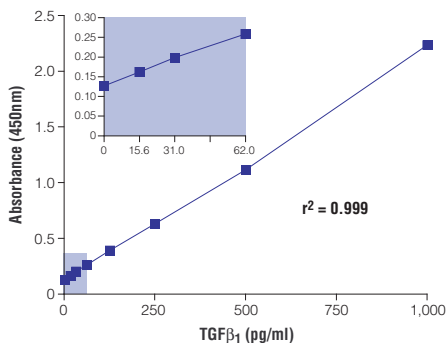
**Caution:** Take care to avoid contact of the TMB One Solution and the 1N hydrochloric acid with skin and eyes.

2. Incubate at room temperature for 15 minutes. Do not shake. A blue color will form in the wells.
3. Stop the reaction by adding 100 $\mu$ l of 1N hydrochloric acid to the wells in the same order in which TMB One Solution was added to the wells in Step 2. Blue will change to yellow upon acidification. Take care to avoid the creation of bubbles.
4. Record the absorbance at 450nm on a plate reader within 30 minutes of stopping the reaction. See Figure 3 for a representative TGF $\beta$ <sub>1</sub> standard curve.

**Note:** The exterior bottom of the plate must be optically clean for accurate measurement. Wipe the **exterior bottom** with 70% ethanol if necessary.

## 5.J. Representative Standard Curve

**!** **Note:** A standard curve must be prepared for each plate.



**Figure 3. Representative TGFβ<sub>1</sub> standard curve obtained using the TGFβ<sub>1</sub> E<sub>max</sub><sup>®</sup> ImmunoAssay System.** The inset is an enlargement of the 0–62.5pg/ml portion of the graph.

## 6. Troubleshooting

### Symptoms

### Causes and Comments

Sample absorbance is above range of standard curve

Sample too concentrated:

- Further dilute the sample.
- Assay multiple dilutions of each sample to ensure that at least one point will fall in the range of the standard curve.

TGFβ<sub>1</sub> present in buffer or medium.  
Perform negative control reactions containing carrier solution but no sample whenever TGFβ<sub>1</sub> may be present in buffer or medium.

Sample absorbance is below range of standard curve

Sample too dilute:

- Reassay at a higher sample concentration.
- Recheck the dilutions of each component of the assay.

Variability in replicate samples

Technique problems in performing the assay:

- Allow the plate to warm to room temperature for 10–15 minutes before starting blocking procedure (Section 5.B).
- Add stop solution to wells in same order as TMB One Solution.
- Change pipette tips before adding each reagent.

## 6. Troubleshooting (continued)

Symptoms	Causes and Comments
Variability in replicate samples (continued)	Technique problems in performing the assay: <ul style="list-style-type: none"> <li>• Perform additional replicates. Ensure that all wells are washed completely.</li> <li>• Check calibration of the pipettor.</li> </ul>
Low activity in TGFβ <sub>1</sub> Standard	Improper storage. The undiluted standard is stable for six months at -20°C and three months at 4°C.

## 7. Reference

1. Hornbeck, P. (1994) Enzyme-linked immunosorbent assays. In: *Current Protocols in Immunology*, Vol. 1, Coico, R., ed., John Wiley & Sons, Inc., Unit 2.1, NY.

## 8. Appendix

### 8.A. Performance Characteristics of the TGFβ<sub>1</sub> E<sub>max</sub><sup>®</sup> ImmunoAssay System

#### Cross-Reactivity of the TGFβ<sub>1</sub> E<sub>max</sub><sup>®</sup> ImmunoAssay System

The TGFβ<sub>1</sub> E<sub>max</sub><sup>®</sup> ImmunoAssay System demonstrates very low cross-reactivity with other TGFβ isoforms.

TGFβ Isoform	Apparent TGFβ <sub>1</sub> Concentration	% Cross-Reactivity
TGFβ <sub>1,2</sub>	1.15ng/ml	11.5%
TGFβ <sub>2</sub>	0.16ng/ml	1.6%
TGFβ <sub>3</sub>	0.07ng/ml	0.7%

10ng/ml of these TGFβ isoforms were diluted in TGFβ Sample 1X Buffer and assayed with this system. **Note:** TGFβ<sub>1,2</sub> is a heterodimer containing a TGFβ<sub>1</sub> and TGFβ<sub>2</sub> chain.

### Effect of Endogenous TGFb1 on Amount of Sample Measured

Due to endogenous TGFb1 in serum and plasma, we recommend acid treating human serum and EDTA plasma samples and then diluting the samples 1:150 in TGFb1 Sample 1X Buffer before assaying.

Sample	Endogenous TGFb <sub>1</sub> (pg/ml)	TGFb <sub>1</sub> Spiked Amount (pg/ml)	Expected TGFb <sub>1</sub> (pg/ml)	Apparent TGFb <sub>1</sub> (pg/ml)
RPMI 1640 +				
10% FBS	142	500	642	717
Serum	148	62	210	239
Plasma	89	62	151	182
Urine	0	125	125	121

Samples containing endogenous TGFb<sub>1</sub> were acid treated, neutralized, diluted and spiked with activated Natural Human TGFb<sub>1</sub>.

### Intra-Assay Comparison

Three concentrations of Natural Human TGFb1 (acid treated) were diluted in TGFb Sample 1X Buffer and assayed by one operator for a total of eight determinations each.

	Low	Medium	High
N	8.0	8.0	8.0
Mean (pg/ml)	132.0	553.0	958.0
SD (pg/ml)	6.0	9.0	32.0
CV (%)	4.5	1.6	3.3

N = sample size, SD = standard deviation,  
CV = coefficient of variance

### Inter-Assay Comparison

Three concentrations of Natural Human TGFb1 (acid treated) diluted in TGFb Sample 1X Buffer were assayed independently by four different operators for a total of 20 determinations.

	Low	Medium	High
N	20	20	20
Mean (pg/ml)	120	568	892
SD (pg/ml)	23	66	68
CV (%)	19.1	11.6	7.6

N = sample size, SD = standard deviation,  
CV = coefficient of variance

## 8.B. Composition of Buffers and Solutions

### 1N hydrochloric acid

Add 82.7ml of concentrated hydrochloric acid to 917.3ml deionized water.

### carbonate coating buffer

0.025M sodium bicarbonate  
0.025M sodium carbonate  
Adjust pH to 9.7 using 1N HCl or 1N NaOH.

**Note:** The carbonate coating buffer should not be stored for longer than one week, as the pH is unstable over longer periods of time.

### Dulbecco's PBS (per liter)

0.2g KCl  
8.0g NaCl  
0.2g  $\text{KH}_2\text{PO}_4$   
1.15g  $\text{Na}_2\text{HPO}_4$   
100mg  $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$   
130mg  $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$

Add room temperature deionized water to a final volume of 1 liter to the KCl, NaCl,  $\text{KH}_2\text{PO}_4$  and  $\text{Na}_2\text{HPO}_4$ . Adjust pH to 7.35 using 1N HCl or 1N NaOH if necessary. Add the  $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$ , mix thoroughly then add the  $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$  and mix thoroughly.

### TBST wash buffer

20mM Tris-HCl (pH 7.6)  
150mM NaCl  
0.05% (v/v) Tween® 20

## 8.C. Related Products

### $E_{\text{max}}^{\circ}$ ImmunoAssay Systems

Product	Size	Cat.#
TGF $\beta_2$ $E_{\text{max}}^{\circ}$ ImmunoAssay System	5 × 96 wells	G7600
BDNF $E_{\text{max}}^{\circ}$ ImmunoAssay Systems	2 × 96 wells	G7610
	5 × 96 wells	G7611
GDNF $E_{\text{max}}^{\circ}$ ImmunoAssay Systems	2 × 96 wells	G7620
	5 × 96 wells	G7621
NGF $E_{\text{max}}^{\circ}$ ImmunoAssay Systems	2 × 96 wells	G7630
	5 × 96 wells	G7631
NT-3 $E_{\text{max}}^{\circ}$ ImmunoAssay Systems	2 × 96 wells	G7640
	5 × 96 wells	G7641

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**8.C. Related Products (continued)****Items Available Separately**

<b>Product</b>	<b>Size</b>	<b>Cat.#</b>
Anti-TGF $\beta$ <sub>1</sub> pAb	100 $\mu$ g	G1221
TGF $\beta$ Sample 10X Buffer	20ml	G1291
TMB One Solution*	100ml	G7431
rhGDNF	5 $\mu$ g	G2781
Anti-GFAP pAb	100 $\mu$ g	G5601
Anti- $\beta$ III Tubulin mAb	100 $\mu$ g	G7121
Anti-VACHT pAb	100 $\mu$ g	G4481

\*For Laboratory Use

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