GE Healthcare

Amersham Transforming Growth Factor – Beta 1 (TGFβ1) Human, Biotrak ELISA System

Product Booklet

Code: RPN2774

Page finder

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2. Handling

2.1. Safety warnings and precautions

Warning: ***For research use only.*** Not recommended or intended for diagnosis of disease in humans or animals. Do not use internally or externally in humans or animals.

All chemicals should be considered as potentially hazardous. We therefore recommend that this product is handled only by those persons who have been trained in laboratory techniques and that it is used in accordance with the principles of good laboratory practice. Wear suitable protective clothing such as laboratory overalls, safety glasses and gloves. Care should be taken to avoid contact with skin or eyes. In the case of contact with skin or eyes wash immediately with water. See material safety data sheet(s) and/or safety statement(s) for specific advice.

Note that the assay protocol requires the use of Glacial Acetic Acid, Hydrochloric Acid and Sodium Hydroxide.

Warning: Acetic Acid (Glacial), Hydrochloric Acid and Sodium Hydroxide are corrosive.

Please follow the manufacturers' safety data sheets relating to the safe handling and use of these materials.

2.2. Storage

Store at 2°C to 8°C.

2.3. Expiry

The expiry date is stated on the package and will normally be at least 4 weeks from the date of despatch.

3. Components

Microplate
The plate contains 12 x 8 well strips coated with mouse anti-TGFβ. Ready for use.

Biotinylated detection antibody
Tube contains polyclonal antibody conjugated to biotin, 75 µl, with Sodium Azide.

Streptavidin-horseradish peroxidase conjugate
Tube contains Streptavidin conjugated to HRP, 50 µl.

Standard
Two bottles containing lyophilized recombinant human $TGF\beta_1$, in an acid/Gelatin buffer which, when reconstituted, gives 21 ng/ml, 0.5 ml on reconstitution.

Standard reconstitution reagent
Bottle contains 2 ml of 4 mM HCl/0.1% BSA and preservative, ready for use.

Assay buffer
Bottle contains 15 ml of assay buffer concentrate which, when diluted, gives a 0.01 M Phosphate Buffered Saline solution pH 7.4 containing 0.1% (w/v) Bovine Serum Albumin (BSA) and preservative, 150 ml on dilution.

Wash buffer
Bottle contains 15 ml of wash buffer concentrate which, when diluted, gives a 0.01 M Phosphate Buffered Saline solution pH 7.4 containing 0.2%(v/v) Tween™20 and preservative, 1500 ml on dilution.

PBS tablets
Bottle contains 9 PBS tablets to provide 1800 ml of 0.01 M Phosphate Buffered Saline pH 7.4 for dilution of the assay and wash buffer concentrates.

TMB substrate
Bottle contains 3,3',5,5'-Tetramethylbenzidine/Hydrogen Peroxide solution, ready for use.

Stop solution
Bottle contains 12 ml of 0.19 M Sulphuric Acid, ready for use.

4. Other materials required

Materials and equipment

The following materials and equipment are required but not supplied:

- Pipettes or pipetting equipment with disposable polypropylene tips, (1 ml, 100 µl, 50 µl and 10 µl)
- Disposable polypropylene test tubes (eg. Röhren 5 ml tubes, Sarstedt code 55 526 and Costar 15 ml tubes, Corning Costar Corporation code 3218)
- Measuring cylinders (2 litre, 250 ml, 10 ml)
- Distilled or deionized water
- 37°C incubator
- Vortex mixer
- Timer
- pH paper (pH 0-14)
- Magnetic stirrer and stirrer bar
- Spectrophotometric plate reader capable of measuring at 450 nm
- Automatic plate washer (optional)
- Serum collector Becton Dickinson Vacutainer™ brand SST™ tubes for serum collection, code 36 7696
- Plasma collector Becton Dickinson Vacutainer brand blood collection tubes (EDTA), code 36 7655
- Refrigerated centrifuge capable of 1000 x g.
- Microcentrifuge capable of 5800 x g.

Reagents for activation step

- Glacial Acetic Acid eg Merck code 10001
- HEPES free acid eg Sigma code H3375
- Hydrochloric Acid (concentrated) eg Merck code 10307
- Sodium Hydroxide pellets eg Merck code 10252

Use reagent or AnalaR grade chemicals

5. Description

- Specific for human TGFβ_1
- Precise measurement
- Non-isotopic protocol with ready to use substrate
- Flexible same day or overnight protocol
- High sensitivity, 4 pg/ml for overnight and same day protocols

The TGFβ_1, Human, Biotrak™ ELISA System from GE Healthcare provides a simple, specific and precise quantitative determination of (h)TGFβ_1. The assay has been specifically designed for research purposes. TGFβ_1 may be measured over the range 15.6 to 1000 pg/ml.

The assay is based on a two site ELISA 'sandwich' format, see figure 1. Standards and samples are incubated in microtitre wells precoated with mouse anti-TGFβ antibody. Any TGFβ present will be bound to the wells, other components of the sample being removed by washing and aspiration. TGFβ_1 is then detected using a biotinylated polyclonal antibody. HRP activity is determined by the addition of TMB substrate solution. The reaction is stopped by addition of an acid solution and the resultant color read at 450 nm in a microplate spectrophotometer. The concentration of TGFβ_1 in a sample is determined by interpolation from a standard curve.

Each pack contains reagents for 96 determinations. This allows the construction of a standard curve plus the measurement of 40 samples in duplicate.

6. Critical parameters

The following points are critical:

- Use only coated wells from the same reagent batch for each assay.
- Allow samples and all reagents to reach room temperature before performing the assay. This is particularly important for the substrate.
- Mix samples and all reagents thoroughly before use but avoid excessive foaming.
- A standard curve must be run on each plate.
- New pipette tips must be used for each standard or sample, which should be assayed in duplicate. GE Healthcare recommends the use of multiple tip or multi-shot dispensing equipment for the addition of other reagents to the wells.
- The total dispensing time for each plate should not exceed 20 minutes.
- Recommended incubation times should be closely followed. If more than one plate is being assayed, each plate should be timed individually. This is particularly important for the substrate step.
- Keep the plates covered with lids during the incubation steps.
- It is particularly important that all wells are washed thoroughly and uniformly. If using an automatic washer, check the head operation before starting the assay. If washing by hand, ensure that all wells are completely filled at each wash. GE Healthcare recommends four wash cycles per wash step.
- Following each wash step, invert the plate and tap several times on absorbent tissue. This should be done vigorously enough to remove any remaining liquid but should not dislodge the strips. Discard the tissues after each wash step.
- Plates should be read within 30 minutes of terminating the enzyme reaction.

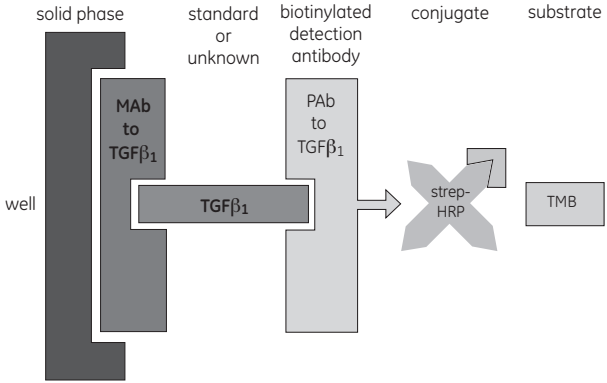

Figure 1 TGFβ_1 ELISA assay design

Figure 1. TGFβ_1 ELISA assay design

7. Specimen collection and sample preparation

Representative procedures are described below for the preparation of cell culture supernatants, serum and plasma samples. However, it remains the responsibility of the investigator to validate the chosen procedure.

Cell culture supernatants

Centrifuge at 1000 x g for 10 minutes at 4°C to remove particulates. Store frozen aliquots of supernatants at -15°C to -30°C. Avoid freeze thaw cycles.

Note: We recommend that serum-free culture media are used for growth of cell cultures as bovine serum may contain significant levels of latent TGFβ1. Where bovine serum is used, prepare a control for measurement of basal levels of the cytokine.

Serum

Use a serum separator tube. Coagulate samples at 37°C for 30 to 60 minutes within 30 minutes of collection. Centrifuge samples at 1000 x g for 10 minutes at 4°C. Remove serum and store frozen aliquots at -15°C to -30°C. Avoid freeze-thaw cycles.

Plasma

Platelets contain high levels of $TGF\beta_1$ which are released on platelet degranulation. The following procedure has been designed to minimize platelet degranulation during blood collection and plasma separation.

Collect plasma using EDTA as an anti-coagulant. We recommend that blood is collected using a syringe and then transferred to a collection vessel containing EDTA. Vacutainers can be used as collection vessels but blood should still be drawn with a syringe to avoid any platelet degranulation resulting from the vacuum.

Remove the syringe needle and the vacutainer lid before transferring the blood. Mix the blood and EDTA by inverting several times and immediately place on ice.

Within 60 minutes of blood collection separate the plasma by centrifugation at 1000 x g for 30 minutes at 4°C. Remove plasma and store frozen aliquots at -15°C to -30°C.

Platelet depleted plasma can be prepared by thawing the plasma samples prepared as described above and then centrifuging at 5800 x g for 10 minutes. Platelet poor plasma is removed from the platelet pellet. We recommend that this procedure is carried out immediately before the activation step described in the next section. In our experience, levels of TGFβ_1 in platelet depleted plasma are around 50% of those obtained from platelet rich plasma.

Note: If samples are likely to be stored for longer than one month before being assayed we would recommend storage at -70°C if possible. Freeze-thaw cycles should be avoided.

Note: The measurement of cytokines in serum and plasma has been reported to be affected by non-specific matrix effects which may vary between samples from different individuals (1–3). Dilution of such samples may help to reduce these interference effects.

8. Sample activation

TGFβs are secreted from cells as latent complexes. To bind to specific cell surface receptors, $TGF\beta_1$ must be activated and released from this latent complex. *In vitro* activation can be accomplished by acid treatment.

Preparation of reagents
Activation of $TGF\beta_1$ produces the immunoreactive form of this cytokine. The following solutions are required for activation and subsequent neutralization of the reaction mixture. Solutions can be stored in glass bottles for up to 1 month at room temperature. Wear the appropriate protection for preparation and use of these reagents.

Reagents for cell culture supernatants
1 M HCl (100 ml) - slowly add 10 ml of concentrated (10 M) HCl to 90 ml of distilled or deionized water with continual mixing.

1.2 M NaOH/0.5 M HEPES (100 ml) - Slowly add 4.8 g NaOH pellets to 75 ml of distilled or deionized water and mix well. Add 11.9 g of HEPES and mix. Adjust the final volume to 100 ml with distilled or deionized water.

Reagents for serum/plasma samples
2.5 M Acetic Acid (250 ml) - Slowly add 35.9 ml of 17.4 M Glacial Acetic Acid to 214 ml of distilled or deionized water and mix well.

2.7 M NaOH/1M HEPES (250 ml) - Add 27 g NaOH pellets to 150 ml of distilled or deionized water and mix well. Add 59.5 g of HEPES to this solution and mix thoroughly. Adjust the final volume to 250 ml with distilled or deionized water.

Activation procedures
It is important to achieve a pH of 1–2 during activation and pH 7–8 after neutralization. Under these conditions, samples are

activated and subsequently assayed at a pH optimal for the ELISA system.

Cell culture supernatants

Due to the possible variation in pH of various cell culture media, it is important to check that the pH values are actually reached. If not, adjust the pH to the appropriate value.

Some sera contain significant levels of latent $TGF\beta_1$, eg bovine serum. Therefore, basal levels in control medium should be determined and subtracted from quantities found in conditioned medium. Cell culture supernatants (with or without serum) are processed as follows:-

1. Add 0.2 ml of 1 M Hydrochloric Acid to 1 ml of conditioned medium. Mix well and incubate for 10 minutes.
2. After 10 minutes at room temperature, neutralize the acidified medium with 0.2 ml of 1.2 M NaOH/0.5 M HEPES free acid and assay using the protocol described on page 18.
3. If bovine serum added as a supplement to conditioned media exceeds 5%, further dilute the activated sample at least 1:2 using assay buffer.

The dilution as a result of the sample activation procedure is (1:1.4).

Serum/plasma

1. Add 0.2 ml of 2.5 M Acetic Acid to 0.2 ml of serum/plasma. Mix well and incubate for 10 minutes.
2. After 10 minutes at room temperature, neutralize the acidified sample with 0.2 ml of 2.7 M NaOH/1 M HEPES free acid.

The dilution as a result of the serum/plasma activation procedure is (1:3). Further dilute serum, platelet rich plasma and platelet poor plasma 1:100, 1:10 and 1:5 respectively using assay buffer. This gives an overall dilution of 1:300 for serum, 1:30 for platelet rich plasma and 1:15 for platelet poor plasma. Assay samples as described on page 18.

Note: These dilutions are designed to produce OD readings towards the lower end of the curve for normal samples. Some pathological samples may have moderately higher levels of $TGF\beta_1$ which can be measured from the standard curve. However, if the levels of $TGF\beta_1$ are markedly elevated, further dilution of the samples is recommended.

Non-activated samples

For determining $TGF\beta_1$ levels in samples that have not been activated, use undiluted samples.

For non-activated serum or plasma samples we recommend that this assay is used in combination with either heterophilic blocking reagent or heterophilic blocking tubes which can be purchased from Scantibodies Laboratory Inc. For further details contact your local GE Healthcare representative.

9. Protocol

9.1. Reagent preparation

- **Note:** All reagents must be allowed to equilibrate to room temperature before preparation. This is particularly important for the enzyme substrate.
- Either distilled or deionized water may be used for reagent preparation.
- The microplate, enzyme substrate, standard reconstitution reagent and stop reagent are supplied ready for use when equilibrated to room temperature.

PBS tablets

Make up 1800 ml of PBS solution by dissolving the 9 PBS tablets provided with distilled water. This solution will be used to prepare both the wash buffer and assay buffer.

Wash buffer

Transfer the contents of the bottle containing wash buffer concentrate to a 2000 ml graduated cylinder by repeated washing with PBS solution. Adjust the final volume to 1500 ml with PBS and mix thoroughly. The diluted wash buffer contains 0.01 M Phosphate Buffered Saline pH 7.4 containing 0.2% Tween 20 and preservative. Store at 2–8°C for up to one month.

Assay buffer

Transfer the contents of the bottle containing assay buffer concentrate to a 250 ml graduated cylinder by repeated washing with PBS solution. Adjust the final volume to 150 ml with PBS and mix thoroughly. The diluted assay buffer contains 0.01 M Phosphate Buffered Saline pH 7.4 containing 0.1% Bovine Serum Albumin and preservative. Store at 2–8°C for up to one month.

TGFβ_1 standard

- Reconstitute the TGFβ_1 standard with 0.5 ml of standard reconstitution reagent. Mix by inverting and vortexing the vial. This reconstitution produces a stock solution of 21 ng/ml. Use this stock to produce a serial 2-fold dilution series, as described in the next section. Use the standard within one hour of reconstitution.

Biotinylated detection antibody, and streptavidin-horseradish peroxidase conjugate

The biotinylated detection antibody and the Streptavidin-Horseradish Peroxidase conjugate are both diluted immediately prior to use at the appropriate stage in the assay protocol. The dilution procedure is described in the assay protocol.

9.2. Preparation of working standards

Note: It is important to use a clean pipette tip for each dilution.

1. Label seven polypropylene tubes 1000, 500, 250, 125, 62.5, 31.3 and 15.6 pg/ml.
2. Pipette 2 ml of assay buffer into the 1000 pg tube and 1 ml into each of the other tubes.
3. Pipette 100 µl from the stock standard vial into the 1000 pg tube.
4. Vortex mix.
5. Pipette 1 ml from the 1000 pg tube into the 500 pg tube.
6. Vortex mix.
7. Repeat this doubling dilution step successively with the remaining tubes.

Note: The reconstituted stock standard and diluted standards should be discarded after use.

9.3. Assay procedure

This ELISA provides the flexibility to run two partial plates on separate

occasions. For such multiple use remove the strips that are not needed for the first assay from the plate frame and place them in the resealable plastic bag with the desiccant provided, ensuring that the bag is tightly sealed. The strips may be stored at 2–8°C for up to 1 month. A second standard vial is also provided to facilitate re-use of the kit.

For recommended sample preparation see page 11.

1. Prepare the reagents as described in 'reagent preparation'.

2. Prepare the working standards as described in the previous section.

3. Set up the microplate with sufficient wells for running all blanks (zero standard), standards and samples as required. We recommend that all standards and samples are assayed in duplicate.

4. Pipette 100 µl of assay buffer into the zero standard wells.

5. Pipette 100 µl of each standard into the appropriate wells.

6. Pipette 100 µl of sample into the appropriate wells.

7. Cover the plate with the lid provided and incubate at 37°C for exactly 1 hour.

8. Aspirate and wash all wells 4 times with wash buffer ensuring that the wells are completely filled and emptied at each wash.

9. After the fourth wash, blot the plate by tapping briskly on tissue paper.

10. Dilute 50 µl of biotinylated detection antibody into 10 ml of assay buffer and mix well. If running a partial plate, dilute sufficient detection antibody stock for the required number of wells (5 ml per ml of assay buffer).

11. Pipette 100 µl of diluted biotinylated detection antibody into each well.

12. Replace the lid and incubate for one hour at 37°C.

13. Repeat the wash and blot as in steps 8 and 9.

14. Dilute 10 µl of the Streptavidin-HRP conjugate in 10 ml of assay

buffer and mix well.

15. Pipette 100 µl of diluted Streptavidin-HRP conjugate into each well.

16. Replace the lid and incubate for 30 minutes at 37°C.

17. Repeat the wash and blot as in steps 8 and 9.

18. Pipette 100 µl of room temperature substrate into all wells.

19. Replace the lid and incubate the plate at room temperature (20–25°C) for 30 minutes.

20. Pipette 100 µl of stop solution into all wells.

21. Read the plates, within 30 minutes of adding stop reagent, at 450 nm.

Note: This assay may also be performed using an overnight incubation at 2–8°C at stage 7 (the standard and sample incubation step).

Note: If running a partial plate, retain the strip holder.

	1	2	3	4	5	6	7	8	9	10	11	12
A	0	0	S1	S1	S9	S9	S17	S17	S25	S25	S33	S33
B	15.6	15.6	S2	S2	S10	S10	S18	S18	S26	S26	S34	S34
C	31.3	31.3	S3	S3	S11	S11	S19	S19	S27	S27	S35	S35
D	62.5	62.5	S4	S4	S12	S12	S20	S20	S28	S28	S36	S36
E	125	125	S5	S5	S13	S13	S21	S21	S29	S29	S37	S37
F	250	250	S6	S6	S14	S14	S22	S22	S30	S30	S38	S38
G	500	500	S7	S7	S15	S15	S23	S23	S31	S31	S39	S39
H	1000	1000	S8	S8	S16	S16	S24	S24	S32	S32	S40	S40

Figure 2. Recommended positioning of standard (0–1000 pg/ml) and sample (S) wells.

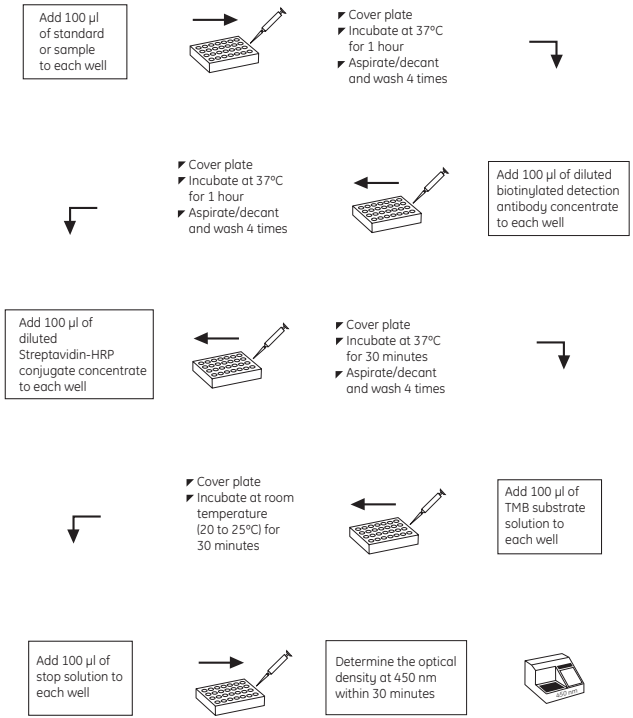

Figure 3. Assay protocol

9.4. Data processing

Calculation of results

1. Typical assay data is shown in table 1.

2. Calculate the mean optical density (OD) for each sample and standard.

3. Subtract the mean OD of the zero standard from each mean value.

4. A linear dose response relationship can be generated by using a log/log plot of (standard OD - zero standard OD) [y axis] against the standard concentration [x axis].

5. A typical standard curve is illustrated in figure 4.

6. Read the values of the samples directly from the graph.

7. Multiply these values by the appropriate dilution factor for each sample (refer to page 15).
For example platelet rich plasma

Sample OD readings	0.253	Mean = 0.250
	0.247	
Zero standard OD readings	0.078	Mean = 0.080
	0.081	

Mean OD - mean NSB = 0.250 - 0.080
Mean OD - mean NSB = 0.170

Value read from graph gives concentration of $TGF\beta_1$ in pg/ml:
= 145 pg/ml

Multiply by dilution factor to give the final concentration of $TGF\beta_1$ in ng/ml.
The recommended dilution factor for platelet rich plasma is 30.

145 x 30 = 4350 pg/ml
145 x 30 = 4.35 ng/ml

If preferred, standard curves can be generated by using a semi-log plot of the mean OD of the standards against the standard concentration. Curves may be drawn using either a smoothed spline computer program or by using a flexicurve and semi-log graph paper.

Typical assay data

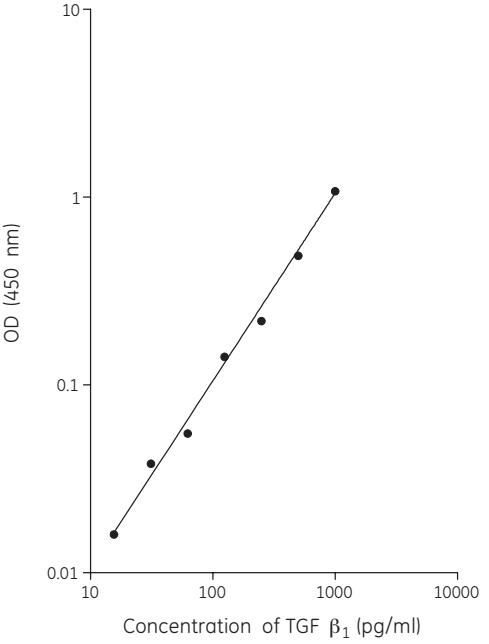

Figure 4. Typical TGFβ_1 standard curve

Table 1. Typical TGFβ_1 assay data

Standard TGFβ_1 (pg/ml)	Optical density at 450 nm	Mean optical density	Mean OD -OD of zero standard
0	0.159	0.163	
	0.167		
15.6	0.177	0.179	0.016
	0.181		
31.3	0.187	0.201	0.038
	0.216		
62.5	0.212	0.218	0.055
	0.223		
125	0.315	0.304	0.141
	0.293		
250	0.372	0.382	0.219
	0.392		
500	0.652	0.650	0.487
	0.648		
1000	1.310	1.239	1.076
	1.168		

Note: Using the alternative overnight assay procedure the optical densities are approximately 30% higher with an optical density of approximately 3.0 for the most concentrated assay standard.

10. Additional information

10.1. Specificity

The specificity of the assay was determined by adding purified cytokines to the system and comparing the apparent measured $TGF\beta_1$ with that of the $TGF\beta_1$ standard. At the highest concentration tested, cross-reactivity was below the detection limit of the assay with all the tested cytokines. The results are shown in table 2.

Table 2. Cross-reactivity data

Analyte	% Cross reactivity
$TGF\beta_1$	100
$TGF\beta_2$	<1
$TGF\beta_3$	<1
Granulocyte colony stimulating factor	<1
Interleukin-2	<1
Interferon-gamma	<1
Tumour necrosis factor-alpha	<1
Interferon-alpha	<1
Interleukin-1α	<1
Interleukin-4	<1
Granulocyte-macrophage colony stimulating factor	<1
Interleukin-6	<1
Interleukin-10	<1
Interleukin-1β	<1
Interleukin-5	<1

10.2. Sample interference

Activation assay

No matrix interference has been observed from plasma samples in the $TGF\beta_1$ activation assay. Plasma samples which have previously

been shown to contain heterophilic antibodies (20 samples), human anti-mouse antibodies (HAMA, 5 samples) and rheumatoid factor (10 samples) were assayed after acid activation. Values in all but two of these samples were within a range 1.5 to 6 ng/ml which is similar to that observed for blood donor samples. Two HAMA samples gave elevated values of 8.6 and 13.5 ng/ml respectively. Pre-treatment of these samples with 1 µg/ml of neutralizing TGFβ antibody reduced the measured $TGF\beta_1$ values by >90%. In summary, we have not been able to demonstrate any matrix interference in the $TGF\beta_1$ activation assay.

Non-activation assay

For the assay of non-activated serum or plasma samples it is recommended that this assay is used in combination with either the heterophilic blocking reagent or the heterophilic blocking tubes which can be purchased from Scantibodies Laboratory Inc. For further details contact your local GE Healthcare representative.

10.3. Precision

Within-assay

The within-assay precision was determined by measuring multiple replicates in the same assay. The results are shown in table 3.

Table 3. Within-assay precision

Control	mean optical density ±SD	% CV	mean concentration ±SD (pg/ml)	% CV	n
High	0.713 ± 0.03	4.2	497.5 ± 16	3.2	4
Low	0.227 ± 0.01	4.4	109 ± 8.0	7.3	4

Between-assay precision

The between-assay precision was determined using the same controls in multiple assays. The results are shown in table 4.

Table 4. Between-assay precision

Control	Mean	% CV concentration ± SD (pg/ml)	n
High	703 ± 71	10.0	4
Low	251 ± 22	8.9	4

Precision profile

A precision profile of the assay was generated by assaying replicates of each of the standards. The results are shown in table 5.

Table 5. Precision profile

Optical density	% CV	Concentration	% CV (pg/ml)	n
1.583	1.3	1000	1.3	8
0.713	2.8	500	2.2	8
0.387	1.3	250	2.6	8
0.231	3.9	125	6.2	8
0.166	7.2	62.5	22.2	7
0.138	2.2	31.3	12.1	7
0.130	3.0	15.6	23.3	8

10.4. Sensitivity

The sensitivity, defined as the concentration on the standard curve equivalent to 2.0 standard deviations above the zero standard, was determined to be 4 pg/ml for both the same day and overnight incubation protocols.

10.5. Assay drift

Forty controls in duplicate were assayed on a single plate using the recommended plate layout on page 19. Minimal assay drift was observed when using both high and low TGFβ_1 controls.

10.6. Parallelism

Normal EDTA plasma and serum samples were diluted in assay buffer after activation. Multiplication of the assay value by the overall dilution factor yields the corrected values for EDTA plasma and serum

Table 6.

Sample	Dilution	pg/ml	Corrected ng/ml
Plasma 1	1 in 75	75.3	5.65
	1 in 150	33.9	5.09
	1 in 300	19.0	5.70
Plasma 2	1 in 75	91.1	6.83
	1 in 150	36.5	5.48
	1 in 300	23.5	7.05
Plasma 3	1 in 75	84.4	6.33
	1 in 150	38.5	5.78
	1 in 300	16.4	4.92
Serum 1	1 in 75	175	13.13
	1 in 150	79.3	11.90
	1 in 300	44.1	13.23

10.7. Correlation

Correlation with NIBSC standard

The NIBSC standard (89/514) was reconstituted according to the manufacturers information. Standard dilutions equivalent to the GE Healthcare curve range were prepared and assayed in the GE Healthcare kit.

11. Troubleshooting guide

Problems	Possible causes
1. Low optical densities	**1.1.** Check the colour of the substrate in the wells. If it is blue add acid to terminate the reaction and develop the correct yellow colour prior to reading.
	1.2. Check reader wavelength.
	1.3. Ensure all reagents have been equilibrated to room temperature before use.
	1.4. Check reagents have been correctly reconstituted.
	1.5. Check reagents have been stored under the recommended conditions.
	1.6. Check incubation times and temperatures.
	1.7. Ensure that the plate is read within 30 minutes of adding the stop reagent.
2. High optical densities	**2.1.** Check point 1.4.
	2.2. Check point 1.6
3. Poor replication	**3.1.** Ensure automatic washers are working correctly, or that each well is completely filled and emptied at every wash step when hand washing.
	3.2. Check pipette calibration.
	3.3. Ensure troughs used with multichannel pipettes are dedicated to individual components.

Problems	Possible causes
	3.4. If splashing occurs when using multishot pipettes, lubricate the pipette barrel.
	3.5. Ensure that no cross contamination occurs by wiping the inside of the lids with a clean tissue after each incubation step.
	3.6. Ensure that plates have been carefully placed into the incubator and the plate reader, to avoid splashing and resultant cross contamination of the wells.
4. Standard replication is good, but the standards do not fit the curve.	**4.** Check standard dilution procedure.
5. High non-specific binding.	**5.** Check point 3.1.
6. Flattening of the curve at high standard concentration.	**6.** This may occur when using the overnight procedure in combination with spectrophotometers which cannot measure above an optical density of 2. Under these conditions we advise incubating the substrate for only 20 minutes.
7. Brown 'precipitate' in wells	**7.** Under conditions where there are very high levels of enzyme activity in the wells, a brown precipitate will appear on addition of acid. This will initially yield a high optical density but this will decay rapidly. This is indicative of a dilution error with one of the detection reagents.

Problems	Possible causes
8. Automatic and manual plate washing	**8.** In GE Healthcare's experience optical densities for the top standard are ≈0.2 units higher with manual washing compared to an automatic plate washer. The 'representative data' quoted in this pack leaflet was obtained with an automated plate washer.
9. Spectrophotometers	**9.** Some older spectrophotometers cannot read above an optical density of ≈2. We would advise terminating the enzyme reaction after 20 minutes if such a spectrophotometer is used to read the plate.

12. Background and references

Transforming growth factor-β (TGFβ) is a stable, multi-functional polypeptide growth factor. To date, five different forms of TGFβ (1-5) have been identified and most evidence suggests that they have similar biological activities at least in established cell lines. *In vivo*, the cytokine is secreted as biologically inactive complex and activation must occur to release the biologically active form, a 25 kDa homodimer. All latent TGFβs can be activated by acid treatment *in vitro*. Most of the TGFβ-like biological activity secreted by cells in culture is exhibited by either $TGF\beta_1$ or $TGF\beta_2$, the former being the most abundant isoform. Most of the currently published reports on the activities of TGFβ have dealt with $TGF\beta_1$ which has been found in highest concentration in human platelets and mammalian bone but is produced by many other cells in small amounts (4). $TGF\beta_1$ is present in platelet granules and is released upon platelet activation. Therefore, to measure circulating levels platelet free plasma should be collected.

The multi-modal nature of this peptide is seen in its ability to stimulate or inhibit cellular proliferation. It affects many functions of nearly all cells and three forms, $TGF\beta_1$, $TGF\beta_2$ and $TGF\beta_3$ are highly conserved between mammalian species, i.e. the amino acid sequence between human and mouse differ by only one residue. Specific receptors for this cytokine have been found on almost all mammalian cells examined and the effect of the molecule varies depending on cell type and growth conditions (5). $TGF\beta_1$ plays a fundamental role in tissue growth and differentiation by involvement in adipogenesis, myogenesis, chondrogenesis, osteogenesis, epithelia cell differentiation and cell function (6). In general, cells of mesenchymal origin appear to be stimulated by $TGF\beta_1$ whereas hepatocytes, T and B lymphocytes, keratinocytes and many epithelia cells are inhibited by the peptide (7). Its broad spectrum of cellular

targets as well as multi-functional actions suggests that it has a pivotal role in many physiological and pathological processes. Because TGFβ_1 has so many potential systemic effects, its synthesis, secretion, activation and clearance must be tightly regulated and defects in this regulation have potential for the production of pathological conditions (4) for example, recent studies demonstrate that TGFβ_1 plays an important early role in directing the immune response to intracellular pathogens and may actually promote the progression of certain infectious diseases.

A number of potential therapeutic situations exist for the topical or systemic application of TGFβs and for inhibiting the inappropriate expression of these peptides. There is potential for wound healing, cartilage and bone repair, suppression of the immune response in autoimmune diseases and transplant rejection. Many other potential therapeutic applications exist and their feasibility awaits further testing (4). This evidence highlights the importance of the biological activities of this cytokine and research into its many modes of action. Determination of activation *in vivo* and regulation of receptors could help us to determine the crucial role it plays in certain disease processes.

13. Related products

Biotrak range of human cytokine ELISA systems

Interleukin-1α [(h)IL-1α]	RPN2750
Interleukin-1β [(h)IL-1β]	RPN2751
Interleukin-2 [(h)IL-2]	RPN2752
Interleukin-4 [(h)IL-4]	RPN2753
Interleukin-5 [(h)IL-5]	RPN2761
Interleukin-6 [(h)IL-6]	RPN2754
Interleukin-8 [(h)]IL-8]	RPN2764
Interleukin-10 [(h)IL-10]	RPN2755
Interleukin-12, p40/70 [(h)IL-12]	RPN2765
Interleukin-12. p70 [(h)IL-12]	RPN2770
Interleukin-13 [(h)IL-13]	RPN2766
Interleukin-16 [(h)IL-13]	RPN2772
Monocyte Chemoattractant Protein-1 [(h)MCP-1]	RPN2769
Macrophage Inflammatory Protein-1α [(h)MIP-1α]	RPN2773
Macrophage Inflammatory Protein-1β [(h)MIP-1β]	RPN2775
RANTES [(h)RANTES]	RPN2775
Granulocyte-Macrophage Colony Stimulating Factor [(h)GM-CSF]	RPN2756
Interferon-Gamma [(h)IFNγ]	RPN2757
Tumour NecrosisFactor-Alpha [(h)TNFα]	RPN2758
Interferon-Alpha [(h)]IFNα]	RPN2759
Vascular Endothelial Growth Factor [(h)]IF	RPN2779

Biotrak range of human cytokine ELISA (5-plate) systems

Interleukin-2 [(h)IL-2]	RPN2803

Biotrak range of high sensitivity human cytokine ELISA systems

Interleukin-1α [(h)IL-1α]	RPN2780
Interleukin-1β [(h)IL-1β]	RPN2781
Interleukin-4 [(h)IL-4]	RPN2783

Interleukin-6 [(h)IL-6]	RPN2784
Interleukin-10 [(h)IL-10]	RPN2785
Granulocyte-Macrophage Colony Stimulating Factor [(h)GM-CSF]	RPN2786
Interferon-Gamma [(h)IFNγ]	RPN2787
Tumour Necrosis Factor-Alpha [(h)TNFα]	RPN2788
Interferon-Alpha [(h)IFNα]	RPN2789

Biotrak range of mouse cytokine ELISA systems

Interleukin-1α [(m)IL-1α]	RPN2719
Interleukin-1β [(m)IL-1β]	RPN2720
Interleukin-2 [(m)IL-2]	RPN2710
Interleukin-4 [(m)IL-4]	RPN2712
Interleukin-5 [(m)IL-5]	RPN2713
Interleukin-6 [(m)IL-6]	RPN2708
Interleukin-11 [(m)IL-11]	RPN2722
Interleukin-12 [(m)IL-12]	RPN2702
Interleukin-12, p40 [(m)IL-12]	RPN2701
Interleukin-12, p70 [(m)IL-12]	RPN2723
Granulocyte-Macrophage Colony Stimulating Factor [(m)GM-CSF]	RPN2716
Interferon-Gamma, [(m)IFNγ]	RPN2707
Monocyte Chemoattractant Protein-1 [(m)MCP-1]	RPN2703
Tumour Necrosis Factor-Alpha [(m)TNFα]	RPN2718
sICAM-1	RPN2721

Biotrak range of rat cytokine ELISA systems

Interleukin-1α [(r)IL-1α]	RPN2735
Interleukin-1β [(r)IL-1β]	RPN2743
Interleukin-2 [(r)IL-2]	RPN2736
Interleukin-4 [(r)IL-4]	RPN2737
Interleukin-6 [(r)IL-6]	RPN2742
Interleukin-10 [(r)IL-10]	RPN2739

Interferon-Gamma [(r)IFNγ]	RPN2741
GRO/CINC-1 [(r)GRO/CINC-1]	RPN2730
Monocyte Chemoattractant Protein-1 [(r)MCP-1]	RPN2740
Tumour Necrosis Factor-Alpha [(r)TNFα]	RPN2734

Range of unlabelled and radiolabelled growth factors and cytokines

Cell proliferation assay system and reagents

Cell Proliferation kit (for immunocytochemical/ immunohistochemical measurement)	RPN20
Monoclonal Anti-Bromodeoxyuridine	RPN202
Cell Proliferation Labelling Reagent	RPN201

RPN201

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