Ultra-sensitive Human TGF-β1 ELISA

For the quantitation of human TGF-β1 concentrations as low as 0.5pg/mL in cell culture supernatants, serum, and plasma.

INTRODUCTION

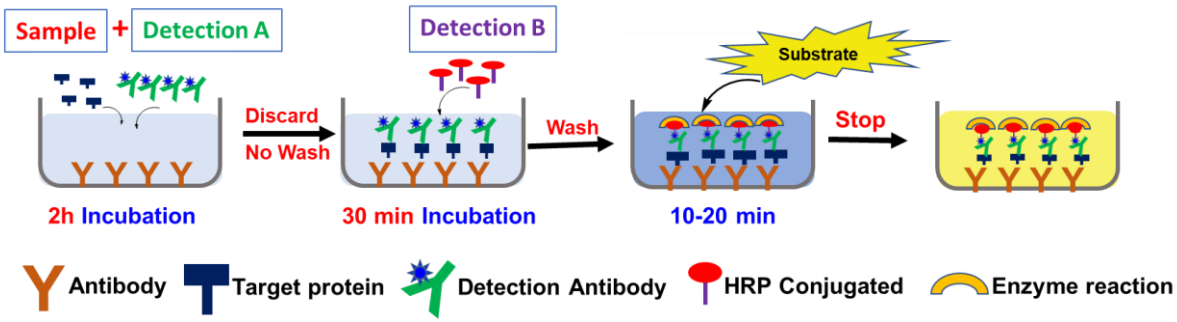
Transforming Growth Factor B (TGF-β) is a stable, multifunctional polypeptide growth factor. TGF-β exists in at least five unique isoforms; TGF-β1, TGF-β2, TGF- β3, TGF-β4, and TGF-β5. TGF-β1 is the prevalent form and is found almost ubiquitously while the other isoforms are expressed in a more limited spectrum of cells and tissues. It is normally secreted as an inactive or latent complex.

The Ultra-sensitive Human TGF-β1 ELISA is a solid phase ELISA to measure low concentration of human TGF-β1 at 0.5pg/mL level in cell culture supernatants, serum, and plasma, which is not possible to be detected with the regular ELISA. The main feature of this the kit is use of a novel approach to replace traditional methods. The kit is designed to ensure short steps, a short incubation time, and only one-step washing out. The measurement can be finished within 2 hours instead of the traditional 4-5 hours. The detection range is between 0.5-2048 pg/mL. The levels of human TGF-β1 samples are parallel to the standard curves obtained using the kit standards linearly. These results indicate that this kit can be used to determine relative mass values for natural human TGF-β1 protein.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for human TGF-β1 was pre-coated onto a microplate. Standards and samples are then pipetted into the wells and incubated with a detection antibody specific for human TGF-β1. Aspirate each well, then add Streptavidin-HRP, incubate the complex. Following a wash to remove any unbound antibody and samples, an ultra-sensitive TMB substrate solution is added to the wells for color develops. The color intensity is proportional to the amount of TGF-β1 bound in the initial step. The intensity of the color is measured by plate read at 450 nm.

Fig. 1 Simple Assay Procedures



KIT CONTENT AND STORAGE CONDITIONS

Part Name	Part#	Description	Storage Conditions
Human TGF-β1 Microplate	TBS3232UA	96-well microplate (12-strips of 8-wells) coated with a Capture Antibody specific for human TGF-β1.	The unused wells can be stored the sealed foil pouch containing the desiccant pack for up to 1 month at 2-8 °C.
Human TGF-β1 Standard	TBS3232UB	25 µL of Recombinant human TGF-β1protein (102.4 ng/mL).	Aliquot and store at -20 °C for up to 6 months in a manual defrost freezer. Avoid repeated freeze-thaw cycles.
Detection A	TBS3232UC	2.2 mL of human TGF-β1antibody.	May be stored for up to 3 months at 2-8 °C.*
Detection B	TBS3232UD	12 mL of Streptavidin-HRP (1x)	
Assay Diluent	TBS3232UE	12 mL of a buffered protein base with preservatives.	
10x Wash Buffer	TBS3000W	12 mL of concentrated solution (10x).	
TMB Substrate	TBS3000T	12 mL of ultra-sensitive TMB substrate.	
Stop Solution	TBS3000S	6 mL of 2 N sulfuric acid.	

Store the unopened kit at 2-8 ℃. Do not use expired kit.
The kit contains sufficient materials to run an ELISA on one 96 well plate.

PRECAUTIONS

Wear protective gloves, clothing, eye, and face protection. Wash hands thoroughly after handling.

REAGENT PREPARATION

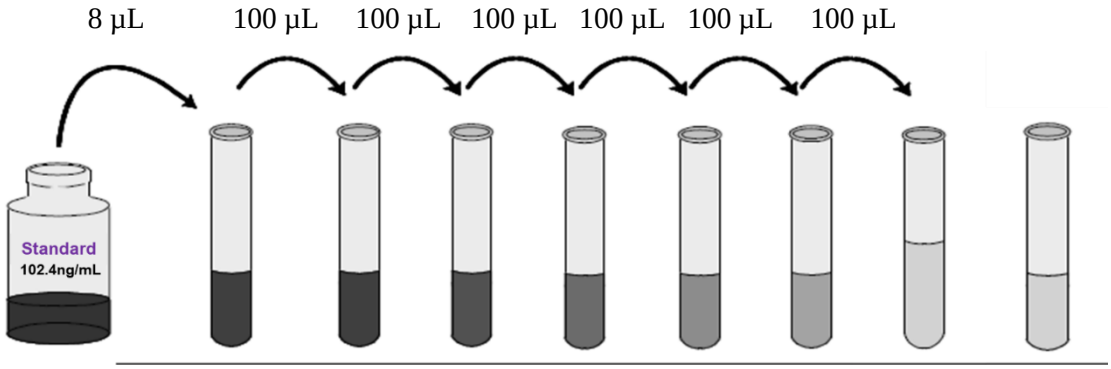
Bring all reagents to room temperature before use.

Wash Buffer: Add 10mL of Wash Buffer Concentrate (10x) to 90mL of deionized distilled water to prepare 100mL of 1xWash Buffer (*If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved.*)

Human TGF-β1 Standard Preparation:

1. Label test tubes 1-8. Pipette 392µL 1x Assay Diluent into tube #1 and 300µL into tubes #2 through #8 as shown in the diagram below (Fig.2).
2. Add 8µL of the Human TGF-β1 Standard stock solution (102.4 ng/mL) to make 2048pg/mL in tube #1. Mix thoroughly.
3. Make 4x serial dilutions of the standard using the 2048 pg/mL standard solution for tubes 2-7 with sequential transfers of 100µL. Mix each tube thoroughly before the next transfer. The standard concentration in tube 1-7 will be 2048, 512, 128, 32, 8, 2 and 0.5 pg/mL respectively. Tube# 8 is Standard 0.

Fig. 2: Standard Preparation



	Std1	Std2	Std3	Std4	Std5	Std6	Std7	Std8
Assay Diluent (µL)	392	300	300	300	300	300	300	300
Addition	Stock	Std1	Std2	Std3	Std4	Std5	Std6	
Vol (µL) of Addition	8	100	100	100	100	100	100	
Std Conc (pg/mL)	2048	512	128	32	8	2	0.5	0

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. Add 80µL of standard, sample, or control per well.
2. Add 20µL of **Detection A** to the above standard and sample of each well, thoroughly mix. Cover with the adhesive sealer. Incubate at **37°C for 2h.**
3. Aspirate each well (***no wash***). Invert the plate and blot it against clean paper towels.
4. Add 100µL of **Detection B** to each well. Incubate at **37°C for 20-30min.**
5. Aspirate each well and wash 3x by filling each well with 300µL Wash Buffer (*Complete removal of liquid at each step is essential to good performance*). After the last wash, remove any remaining Wash Buffer by aspiration or decanting. Invert the plate and blot against clean paper towels.
6. Add 100µL of **TMB Substrate** to each well. Incubate **at RT for 10-20min** (*Shielded from light*). The color will change to blue. If the color is light, the incubation time can be longer.
7. Add 50µL of **Stop Solution** to each well. The color in the well should change from blue to yellow (gently tap the plate to ensure thorough mixing).

8. Determine the optical density of each well within 5 minutes using a microplate reader at 450 nm. If wavelength correction is available, set to 540nm or 570nm. If wavelength correction is unavailable, subtract readings at 540nm or 570nm from the readings at 450nm. This subtraction will correct for optical imperfections in the plate. Readings made directly at 450 nm without correction may be higher and less accurate.

CALCULATION OF RESULTS

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

Create a standard curve 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-β1 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.

TYPICAL DATA

This standard curve is provided for demonstration only (Fig.3). A standard curve should be generated for each set of samples assayed.

SENSITIVITY

The minimum detectable dose (MOD) of human TGF-β1 is typically 0.5 pg/ml.

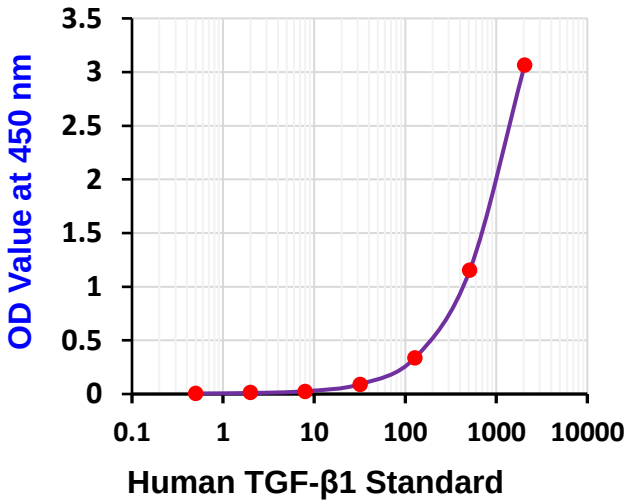
SPECIFICITY

This assay recognizes natural and recombinant human TGF-β1.

RELATIVE PRODUCTS

Human IL-1ß ELISA (TBS3219)
Human IL-2 ELISA (TBS3220)
Human IL-4 ELISA (TBS3221)
Human IL-6 ELISA (TBS3223)
Human IL-7 ELISA (TBS3224)
Human IL-8 ELISA (TBS3225)
Human IL-10 ELISA (TBS3226)
Human IL-13 ELISA (TBS3227)
Human IL-17 ELISA (TBS3228)
Human IL-22 ELISA (TBS3229)
Human IFN-gamma ELISA (TBS3230)
Human GM-CSF ELISA (TBS3233)
Human MIP-1α ELISA (TBS3234)
Human TNF-α ELISA (TBS3235)
Human IL-18 ELISA (TBS3239)

Fig.3 Typical Standard Curve



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