RayBiotech
Empowering your proteomics

3607 Parkway Lane, Suite 200
Peachtree Corners, GA 30092
www.RayBiotech.com | info@raybiotech.com
770-729-2992 | 1-888-494-8555
ISO 13485:2016 cGMP

Rat TGF-beta 1 ELISA Kit

Catalog Number: ELR-TGFb1

Manual
Last Revised: 05/30/2025

Protocol Video

Introduction

Transforming Growth Factor Beta (TGF-beta) is a stable, multifunctional polypeptide growth factor. TGF-beta exists in at least five isoforms, known as TGF-beta 1, TGF-beta 2, TGF-beta 3, TGF-beta 4, TGF-beta 5. Their amino acid sequences display homologies on the order of 70-80%. The various TGF-beta isotypes share many biological activities and their actions on cells are qualitatively similar in most cases although there are a few examples of distinct activities. TGF-beta 1 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 RayBio® Rat TGF-beta 1 ELISA (Enzyme-Linked mmunosorbent Assay) kit is an in vitro enzyme-linked immunosorbent assay for the quantitative measurement of Rat TGF-beta 1 in serum, plasma, and cell culture supernatants. This assay employs an antibody specific for Rat TGF-beta 1 coated on a 96-well plate. Standards and samples are pipetted into the wells and TGF-beta 1 present in a sample is bound to the wells by the immobilized antibody. The wells are washed and biotinylated anti- Rat TGF-beta 1 antibody is added. After washing away unbound biotinylated antibody, HRP-conjugated streptavidin is pipetted to the wells. The wells are again washed, a TMB substrate solution is added to the wells and color develops in proportion to the amount of TGF-beta 1 bound. The Stop Solution changes the color from blue to yellow, and the intensity of the color is measured at 450 nm.

Short on sample, or need higher sensitivity? Check out the IQELISA® Immuno-PCR assay platform and our Simoa® Single Molecule Protein Detection Services.

Storage

The entire kit may be stored at -20°C for up to 1 year from the date of shipment. Avoid repeated freeze-thaw cycles. The kit may be stored at 4°C for up to 6 months. For extended storage, it is recommended to store at -80°C. For prepared reagent storage, see table below.

Reagents

Name	Catalog #	Size / Qty	Description	Storage / Stability After Preparation
Rat TGF beta 1 Microplate	ELR-TGFb1-A	1 Plate	96 wells (12 x 8) coated with anti-Rat TGF beta 1	1 month at 4°C*
Rat TGF beta 1 Standard Protein	ELR-TGFb1-STD	2 vials	Each vial is enough to run each standard in duplicate	1 week at -80°C
Rat TGF beta 1 Detection Antibody	ELR-TGFb1-F	2 vials	Each vial is enough to assay half the microplate	5 days at 4°C
Wash Buffer	EL-ITEMB	25 mL	20X concentrated solution	1 month at 4°C
HRP-Streptavidin	EL-HRP	200 µL	400X concentrated HRP-conjugated streptavidin	Do not store and reuse
TMB One-Step Substrate Reagent	EL-TMB	12 mL	3,3,5,5'-tetramethylbenzidine in buffer solution	N/A
Stop Solution	EL-STOP	8 mL	0.2 M sulfuric acid	N/A
Assay Diluent B	EL-ITEME	15 mL	5X concentrated diluent buffer	1 month at 4°C
96-Well Plate Sealing Film	EL-FILM	2 films	Optional use for overnight incubations	N/A

*Return unused wells to the pouch containing desiccant pack, reseal along entire edge.

Additional Materials Required

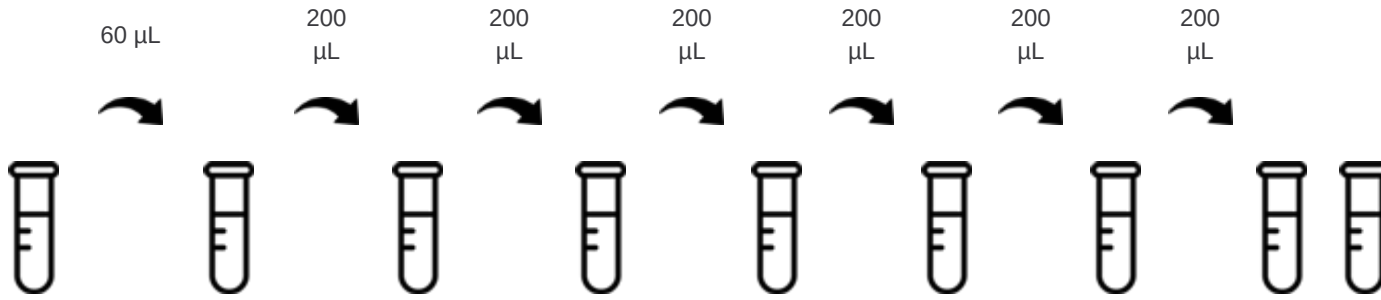
- Microplate reader capable of measuring absorbance at 450 nm
- Precision pipettes to deliver 2 µL to 1 mL volumes
- Adjustable 1-25 mL pipettes for reagent preparation
- 100 mL and 1 L graduated cylinders
- Absorbent paper
- Distilled or deionized water
- Log-log graph paper or computer and software for ELISA data analysis
- Tubes to prepare standard or sample dilutions

Reagent Preparation

1. Bring all reagents and samples to room temperature (18 - 25°C) before use.
2. 5X Assay Diluent B should be diluted 5-fold with deionized or distilled water before use.
3. Sample dilution: 1x Assay Diluent B should be used for dilution of serum/plasma/cell culture supernatants. The suggested dilution for normal serum/plasma is 80 fold after treatment (see activation steps on page 4).

Note: Levels of TGF beta 1 may vary between different samples. Optimal dilution factors for each sample must be determined by the investigator.

4. Preparation of standard: Briefly spin a vial of standard protein. Add 400 µL 1X Assay Diluent B (should be diluted 5-fold with deionized or distilled water before use) into the Standard Protein vial to prepare 25 ng/mL standard. Dissolve the powder thoroughly by a gentle mix. Add 60 µL 25 ng/mL TGF-beta 1 standard from the vial of Standard Protein, into a tube with 940 µL 1X Assay Diluent B to prepare a 1500 pg/mL standard solution. Pipette 300 µL 1x Assay Diluent B into each tube. Use the 1500 pg/mL standard solution to produce a dilution series (shown below). Mix each tube thoroughly before the next transfer. 1x Assay Diluent B serves as the zero standard (0 pg/mL).



		Std 1	Std 2	Std 3	Std 4	Std 5	Std 6	Std 7	Zero Std
Diluent volume	Standard + 400 µL	940 µL	300 µL	300 µL	300 µL	300 µL	300 µL	300 µL	300 µL
Conc.	25 ng/mL	1500.000 pg/mL	600.000 pg/mL	240.000 pg/mL	96.000 pg/mL	38.400 pg/mL	15.360 pg/mL	6.140 pg/mL	0.00 pg/m

5. If the Wash Buffer (20X) contains visible crystals, warm to room temperature and mix gently until dissolved. Dilute 20 mL of Wash Buffer into deionized or distilled water to yield 400 mL of 1X Wash Buffer.
6. Briefly spin the Detection Antibody vial before use. Add 100 µL of 1X Assay Diluent B into the vial to prepare a detection antibody concentrate. Pipette up and down to mix gently (the concentrate can be stored at 4°C for 5 days). The detection antibody concentrate should be diluted 80-fold with 1X Assay Diluent B and used in step 5 of Part VI Assay Procedure.
7. Briefly spin the HRP-Streptavidin concentrate vial and pipette up and down to mix gently before use, as precipitates may form during storage. HRP-Streptavidin concentrate should be diluted 400-fold with 1X Assay Diluent B.

   For example: Briefly spin the HRP-Streptavidin vial and pipette up and down to mix gently. Add 20 µL of HRP-Streptavidin concentrate into a tube with 10 ml 1X Assay Diluent B to prepare a 500-fold diluted HRP-Streptavidin solution (don't store the diluted solution for next day use). Mix well.

***Reagents to activate serum, plasma, and cell culture supernatant samples**

1 N HCl (100 ml) – Slowly add 8.33 ml of 12 N HCl into 91.67 ml deionized water. Mix bottle.
1.2 N NaOH/0.5 M HEPES (100 ml) - Slowly add 12 ml of 10 N NaOH into 75 ml deionized water. Mix bottle. Add 11.9 g HEPES. Mix through. Bring final volume to 100 mL with deionized water.

TGF-beta 1 SAMPLE ACTIVATION PROCEDURE
To activate latent TGF-beta 1 to the immunoreactive form, follow the activation procedure outlined below. Assay

samples after neutralization (pH 7.0 - 7.6). Use polypropylene test tubes.
Notes: Do not activate the kit standards. The kit standards contain active TGF-beta 1.

1. Cell Culture Supernates
Add 0.1 ml 1 N HCI into 0.5 mL cell culture supernates. Mix tube thoroughly. Incubate for 10 minutes at room temperature. Neutralize the acidified sample by adding 0.1 ml 1.2 N NaOH/0.5 M HEPES (PH=7.0~7.6). Mix tube thoroughly. Assay immediately. The activated sample may be diluted with 1X Assay Diluent B. The concentration read off the standard curve must be multiplied by the dilution factor.

2. Serum/plasma
Add 0.05 ml 1 N HCI to 0.1 ml serum. Mix tube thoroughly. Incubate for 10 minutes at room temperature. Neutralize the acidified sample by adding 0.05 ml 1.2 N NaOH/0.5 M HEPES (PH=7.0~7.6). Mix tube thoroughly. Assay immediately. The activated sample may be diluted with 1X Assay Diluent B. The concentration read off the standard curve must be multiplied by the dilution factor.

Assay Procedure

1. Bring all reagents and samples to room temperature (18 - 25°C) before use. It is recommended that all standards and samples be run at least in duplicate.
2. Label removable 8-well strips as appropriate for your experiment.
3. Add 100 µL of each standard (see Reagent Preparation step 4) and sample into appropriate wells. Cover wells and incubate for 2.5 hours at room temperature with gentle shaking.
4. Discard the solution and wash 4 times with 1X Wash Solution. Wash by filling each well with Wash Buffer (300 µL) using a multi-channel Pipette 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.
5. Add 100 µL of 1X prepared biotinylated antibody (Reagent Preparation step 6) to each well. Incubate for 1 hour at room temperature with gentle shaking.
6. Discard the solution. Repeat the wash as in step 4.
7. Add 100 µL of prepared Streptavidin solution (see Reagent Preparation step 7) to each well. Incubate for 45 minutes at room temperature with gentle shaking.
8. Discard the solution. Repeat the wash as in step 4.
9. Add 100 µL of TMB One-Step Substrate Reagent to each well. Incubate for 30 minutes at room temperature in the dark with gentle shaking.
10. Add 50 µL of Stop Solution to each well. Read at 450 nm immediately.

Assay Procedure Summary

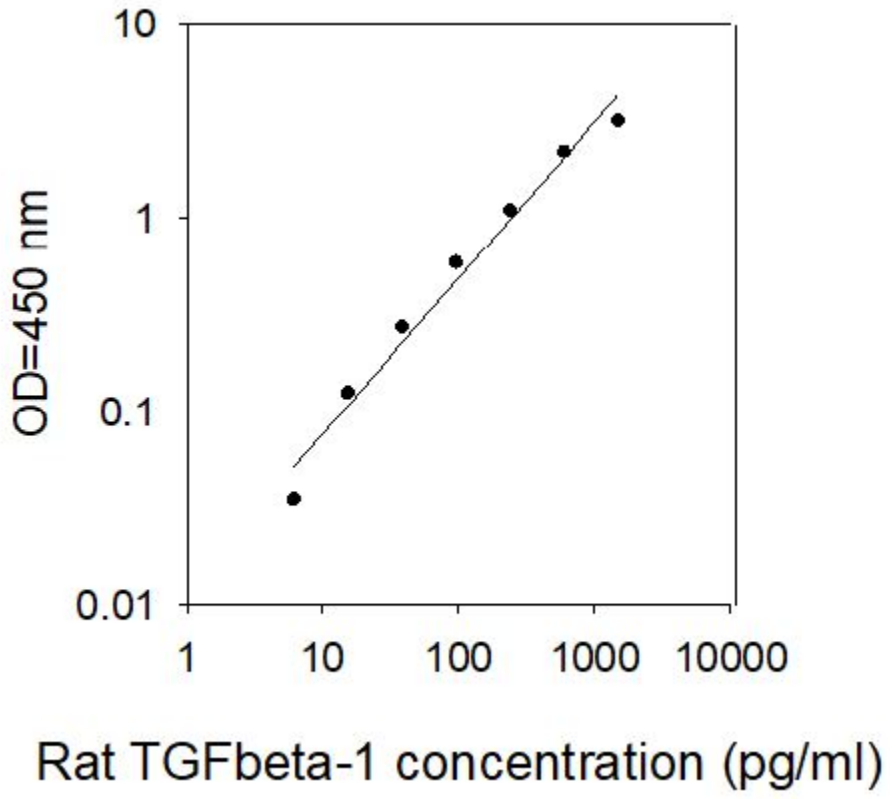
1. Prepare all reagents, samples and standards as instructed.
2. Add 100 µL standard or sample to each well. Incubate 2.5 hours at room temperature.
3. Add 100 µL prepared biotin antibody to each well. Incubate 1 hour at room temperature.
4. Add 100 µL prepared Streptavidin solution. Incubate 45 minutes at room temperature.
5. Add 100 µL TMB One-Step Substrate Reagent to each well. Incubate 30 minutes at room temperature.
6. Add 50 µL Stop Solution to each well. Read at 450 nm immediately.

Calculation of Results

Calculate the mean absorbance for each set of duplicate standards, controls and samples, and subtract the average zero standard optical density. Plot the standard curve on log-log graph paper or using Sigma plot software, with standard concentration on the x-axis and absorbance on the y-axis. Draw the best-fit straight line through the standard points.

Typical Data

These standard curves are for demonstration only. A standard curve must be run with each assay.



Sensitivity

The minimum detectable dose of Rat TGF beta 1 was determined to be 3.5 pg/ml.

Minimum detectable dose is defined as the analyte concentration resulting in an absorbance that is 2 standard deviations higher than that of the blank (diluent buffer).

Spiking & Recovery

Recovery was determined by spiking various levels of Rat TGF beta 1 into the sample types listed below. Mean recoveries are as follows:

Sample Type	Average % Recovery	Range (%)
Serum	78.43	72-82
Plasma	74.68	71-81
Cell culture media	88.17	71-102

Linearity

Sample Type		Serum	Plasma	Cell culture media
1:2	Average % of Expected	112.1	106.8	106.9
	Range (%)	108-116	97-114	103-111
1:4	Average % of Expected	128.5	131.8	128.0
	Range (%)	123-134	124-137	125-131

Reproducibility

Intra-Assay CV%: <10%

Inter-Assay CV%: <12%

Specificity

This ELISA antibody pair detects Rat, Mouse and Human TGF-beta-1. Other species not determined.

Troubleshooting Guide

Problem	Cause	Solution
Poor standard curve	• Inaccurate pipetting • Improper standard dilution	• Check pipettes • Briefly centrifuge the standard protein and dissolve the powder thoroughly by gently mixing
Low signal	• Improper preparation of standard and/or biotinylated antibody • Too brief incubation times • Inadequate reagent volumes or improper dilution	• Briefly spin down vials before opening. Dissolve the powder thoroughly. • Ensure sufficient incubation time. Assay procedure step 3 may be done overnight at 4°C with gentle shaking (note: may increase overall signals including background). • Check pipettes and ensure correct preparation
Large CV	• Inaccurate pipetting • Air bubbles in wells	• Check pipettes • Remove bubbles in wells
High background	• Plate is insufficiently washed • Contaminated wash buffer	• Review the manual for proper wash. If using a plate washer, ensure that all ports are unobstructed. • Make fresh wash buffer
Low sensitivity	• Improper storage of the ELISA kit • Stop solution	• Store your standard at <-70°C after reconstitution, others at 4°C. Keep substrate solution protected from light. • Add stop solution to each well before reading plate