TECHNICAL MANUAL

Horse Transforming Growth Factor Beta-1 (TGFB1) ELISA Kit

- **SKU CODE:** EACO0003
- **SIZE:** 96 Assays
- **DETECTION PRINCIPLE:** Sandwich
- Research-Use-Only

Revised: 09/26

Horse Transforming Growth Factor Beta-1 (TGFB1) ELISA Kit

Please read entire manual carefully before starting experiment. DO NOT mix reagents and use reagents from different kits or batches to prevent assay failure.

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1. Key Features

Detection Method:

Sandwich

Sample Type:

Serum, Plasma

Reactivity:

Equus caballus (Horse)

Range:

62.5–4000 pg/mL

Sensitivity:

15.6 pg/mL

2. Storage & Expiry

Assay Genie ELISA Kits are shipped on gel packs. Please store this ELISA kit and/or its components as described in Section 4. The shelf life of this kit is 6 months; the date of expiration is printed on the ELISA box label.

3. Product Description

The Assay Genie Horse Transforming Growth Factor Beta-1 (TGFB1) ELISA Kit is a highly sensitive assay for the quantitative determination of TGFB1 concentrations in horse serum and plasma.

This kit utilises a sandwich enzyme-linked immunosorbent assay (ELISA) format. An antibody specific for TGFB1 is pre-coated onto the wells of a 96-well microplate. Standards and samples are added to the wells, allowing any TGFB1 present to bind to the immobilised antibody. After incubation, unbound substances are removed by washing. A biotin-conjugated detection antibody specific for TGFB1 is then added, which binds to the captured target. Following a wash to remove excess detection antibody, avidin-conjugated horseradish peroxidase (HRP) is introduced, forming a biotin–avidin–HRP complex. After a further washing step, TMB substrate is added to initiate a colorimetric reaction catalysed by HRP, producing a blue product that turns yellow upon addition of the acidic Stop Solution.

The optical density (OD) is measured at 450 nm using a microplate reader. The OD450 value is directly proportional to the concentration of TGFB1 in the sample, which can be determined by reference to a standard curve.

Note: This assay measures total TGFB1. Latent TGFB1 must first be converted to the immunoreactive form using the acid-activation procedure described in Section 7.

4. Kit Contents

Unopened kit: store at 2–8 °C. The storage conditions listed below apply to opened components (provided this is within the expiration date of the kit).

No	Component	Quantity	Storage (opened)
1	Assay plate	1 (96 wells)	2–8 °C; keep sealed in a foil bag with desiccant, avoid damp
2	Standard (freeze-dried)	2	2–8 °C (1 month); -20 °C for longer storage
3	Biotin-antibody (100× concentrate)	1 × 120 µL	2–8 °C
4	HRP-avidin (100× concentrate)	1 × 120 µL	2–8 °C
5	Biotin-antibody Diluent	1 × 15 mL	2–8 °C
6	HRP-avidin Diluent	1 × 15 mL	2–8 °C
7	Sample Diluent	1 × 50 mL	2–8 °C
8	Wash Buffer (25× concentrate)	1 × 20 mL	2–8 °C
9	TMB Substrate	1 × 10 mL	2–8 °C
10	Stop Solution	1 × 10 mL	2–8 °C
11	1 N HCl	1 × 10 mL	Room temperature
12	1.2 N NaOH / 0.5 M HEPES	1 × 10 mL	2–8 °C
13	Adhesive Strip (for 96 wells)	4	-
14	Instruction manual	1	-

Additional materials required:

1. 37 °C incubator (± 0.5 °C).
2. Microplate reader capable of measuring absorbance at 450 nm (correction at 540 nm or 570 nm).
3. Squirt bottle, manifold dispenser, or automated microplate washer.
4. Absorbent paper for blotting the plate.
5. 100 mL and 500 mL graduated cylinders.
6. Precision pipettes and disposable pipette tips.
7. Deionised or distilled water.
8. Polypropylene test tubes for dilution and sample activation.

5. Precautions

1. This kit is for research purposes only and not for diagnostic or therapeutic uses.
2. Store all components as listed in this manual. Do not use the kit after its expiration date.
3. Allow all reagents and samples to reach room temperature before use.
4. Do not mix or substitute reagents with those from different lots or sources.
5. The Stop Solution is an acid solution. Wear eye, hand, face and clothing protection when using it.
6. Complete removal of liquid at each wash step is essential for good performance; do not let the wells dry at any time during the assay.

6. Assay Summary

1. Prepare reagents, samples and standards as instructed.
2. Add 100 µL standard or sample to each well. Incubate 2 hours at 37 °C.
3. Remove the liquid from each well; do not wash.
4. Add 100 µL Biotin-antibody (1×) to each well. Incubate 1 hour at 37 °C.
5. Aspirate and wash 3 times.
6. Add 100 µL HRP-avidin (1×) to each well. Incubate 1 hour at 37 °C.
7. Aspirate and wash 5 times.
8. Add 90 µL TMB Substrate to each well. Incubate 15–30 minutes at 37 °C, protected from light.
9. Add 50 µL Stop Solution to each well. Read at 450 nm within 5 minutes.

7. Sample Preparation

Serum: Use a serum separator tube (SST) and allow samples to clot for two hours at room temperature or overnight at 4 °C before centrifuging for 15 minutes at 1000 × g. Remove serum and assay immediately, or aliquot and store at -20 °C or -80 °C. Avoid repeated freeze-thaw cycles.

Plasma: Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge for 15 minutes at 1000 × g at 2–8 °C within 30 minutes of collection. Assay immediately, or aliquot and store at -20 °C or -80 °C. Avoid repeated freeze-thaw cycles.

Sample notes:

- Samples to be used within 5 days may be stored at 2–8 °C; otherwise store at -20 °C (≤1 month) or -80 °C (≤2 months).
- Grossly haemolysed samples are not suitable for use in this assay.
- If the sample type is not indicated in this manual, perform a preliminary experiment to determine the validity of the kit.

- Predict the concentration before assaying; if values fall outside the standard-curve range, determine the optimal sample dilution.
- Fresh samples without long-term storage are recommended.

Sample Activation Procedure

To activate latent TGFB1 to the immunoreactive form detectable by this assay, follow the acid-activation and neutralisation procedure below (reagents provided in the kit). Assay samples after neutralisation (pH 7.2–7.6). Use polypropylene test tubes.

1. Add 20 µL of 1 N HCl to 80 µL of serum or plasma sample. Mix well.
2. Incubate 10 minutes at room temperature.
3. Neutralise the acidified sample by adding 16 µL of 1.2 N NaOH / 0.5 M HEPES.
4. Mix well and assay immediately.

8. Standard and Reagent Preparation

Bring all reagents to room temperature (18–25 °C) for 30 minutes before use. Prepare fresh standard for each assay and use within 4 hours. Use graduated containers to prepare reagents; do not prepare reagents directly in the Diluent vials. Distilled water is recommended for reagent preparation. Do not make serial dilutions directly in the wells.

1. Biotin-antibody (1×): Centrifuge the vial before opening. Dilute 100-fold (e.g. 10 µL of Biotin-antibody + 990 µL of Biotin-antibody Diluent).

2. HRP-avidin (1×): Centrifuge the vial before opening. Dilute 100-fold (e.g. 10 µL of HRP-avidin + 990 µL of HRP-avidin Diluent).

3. Wash Buffer (1×): If crystals have formed in the concentrate, warm to room temperature and mix until dissolved. Dilute 20 mL of Wash Buffer Concentrate (25×) into distilled water to prepare 500 mL of Wash Buffer (1×).

4. Standard: Centrifuge the standard vial at 6000–10000 rpm for 30 s. Reconstitute the Standard with 1.0 mL of Sample Diluent (do not substitute) to produce a 4000 pg/mL stock solution. Mix and allow to sit for at least 15 minutes with gentle agitation before making dilutions.

Standard dilution: Pipette 250 µL of Sample Diluent into each tube (S0–S6). Use the stock solution to produce a 2-fold dilution series (below), mixing each tube thoroughly before the next transfer. The undiluted Standard serves as the high standard (4000 pg/mL); Sample Diluent serves as the zero standard (0 pg/mL).

Tube	S7	S6	S5	S4	S3	S2	S1	S0
pg/mL	4000	2000	1000	500	250	125	62.5	0

9. Assay Procedure

Bring all reagents and samples to room temperature before use. Centrifuge samples again after thawing. It is recommended that all samples and standards be assayed in duplicate.

1. Prepare all reagents, working standards and samples as directed in the previous sections.

2. Refer to the assay layout sheet to determine the number of wells required; return any remaining wells and the desiccant to the pouch, reseal, and store unused wells at 4 °C.

3. Add 100 µL of standard or sample per well. Cover with the adhesive strip provided. Incubate 2 hours at 37 °C.

4. Remove the liquid from each well; do not wash.

5. Add 100 µL of Biotin-antibody (1×) to each well. Cover with a new adhesive strip. Incubate 1 hour at 37 °C. (Biotin-antibody (1×) may appear cloudy; warm to room temperature and mix until uniform.)

6. Aspirate each well and wash, repeating twice for a total of three washes. Fill each well with 200 µL of Wash Buffer and let it stand for 2 minutes; complete removal of liquid at each step is essential. After the last wash, remove any remaining Wash Buffer, invert the plate and blot against clean paper towels.

7. Add 100 µL of HRP-avidin (1×) to each well. Cover with a new adhesive strip. Incubate 1 hour at 37 °C.

8. Repeat the aspiration/wash process five times as in step 6.

9. Add 90 µL of TMB Substrate to each well. Incubate 15–30 minutes at 37 °C, protected from light.

10. Add 50 µL of Stop Solution to each well and gently tap the plate to ensure thorough mixing.

11. Determine the optical density of each well within 5 minutes using a microplate reader set to 450 nm. If correction is available, set it to 540 nm or 570 nm and subtract the 540/570 nm reading from the 450 nm reading.

10. Data Analysis

Average the duplicate readings for each standard, control and sample, then subtract the mean optical density of the zero standard. Construct a standard curve using software capable of generating a four-parameter logistic (4-PL) curve fit. Alternatively, plot the mean absorbance of each standard on the x-axis against concentration on the y-axis and draw a best-fit curve; the data may be linearised by plotting the log of the TGFB1 concentration versus the log of the OD, with the best-fit line determined by regression analysis (an adequate but less precise fit).

Note: If the samples were diluted, multiply the concentration read from the standard curve by the dilution factor to obtain the concentration before dilution.

11. Typical Data

Standard Curve

The OD values of the standard curve vary according to the conditions of the actual assay (e.g. operator, pipetting technique or temperature). Each user should therefore generate their own standard curve for every assay using the standards supplied. Intra-assay precision is CV < 8% and inter-assay precision is CV < 10%.

12. ELISA Troubleshooting

Problem	Possible Causes	Solutions
Standard curve without signal	Incorrect reagent order; mixed components from different kits; missing reagents.	Ensure correct reagent order and use components from the same kit. Verify all reagents are added.
Overflow OD	Mixed components from different kits; over-concentrated working solution.	Use correct components and prepare solutions at recommended concentrations.
Poor standard curve	Incorrect curve-fitting model.	Try alternative curve-fitting models.
Samples without signal	Sample concentration too low; incompatible buffer; incorrect preparation; sample degradation or excessive freeze-thaw.	Reduce dilution or concentrate the sample. Check buffer compatibility and follow proper preparation and storage.
High CV%	Precipitate formation; unclean plate; foaming; uneven washing; incomplete reagent mixing; pipetting inconsistency.	Dilute samples if needed, avoid foaming, ensure uniform washing, mix reagents thoroughly, and use calibrated pipettes.
Low standard signal	Improperly reconstituted or degraded standards; incorrect pipetting; expired kit; improper storage; over-dried wells.	Reconstitute standards properly, use fresh kits, follow storage recommendations, and prevent wells from drying.
Slow colour development	TMB not equilibrated; incorrect reader wavelength; over-washing.	Pre-warm TMB (30 min at 37 °C), confirm correct wavelength (450 nm), and follow recommended washing times.
High background	Insufficient washing; contaminated wash buffer; excess detection reagents; delayed reading; TMB exposed to light.	Wash adequately, prepare fresh wash buffer, use correct reagent amounts, read results promptly, and incubate TMB in the dark.

Assay Genie 100% money-back guarantee!

If you are not satisfied with the quality of our products and our technical team cannot resolve your problem, we will give you 100% of your money back.

Manufacturers Statement: This final kit system is assembled and quality-released by Assay Genie Limited.