GSI Equine TGFβ1 ELISA Kit- DataSheet

TGFβ1 (transforming growth factor beta 1) was first identified in human platelets as a protein with a molecular mass of 25 kilodaltons with a potential role in wound healing (1). TGFB1, TGFB2, and TGFB3 all function through the same receptor signaling systems. They are members of the large TGFβ superfamily. TGFβ proteins are highly pleiotropic cytokines that regulate processes such as immune function, proliferation and epithelialmesenchymal transition (2-4). It was later characterized as a large protein precursor (containing 390 amino acids) that was proteolytically processed to produce a mature peptide of 112 amino acids (5).

TGFβ activation from latency is controlled both spatially and temporally, by multiple pathways that include actions of proteases such as plasmin and MMP9, and/or by thrombospondin 1 or selected integrins (5, 6). Although different isoforms of TGFβ are naturally associated with their own distinct LAPs, the TGFβ1 LAP is capable of complexing with, and inactivating, all other Equine TGFβ isoforms and those of most other species (7). Mutations within the LAP are associated with CamuratiEngelmann disease, a rare sclerosing bone dysplasia characterized by inappropriate presence of active TGFβ1 (8).

References

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7. Miller, D.M. et al. (1992) Mol. Endocrinol. 6:694.
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PRINCIPLE OF THE ASSAY

This is a quick ELISA assay that reduces time to 50% compared to the conventional method, and the entire assay only takes 3 hours. This assay employs the quantitative sandwich enzyme immunoassay technique and uses biotin-streptavidin chemistry to improve the performance of the assays. An antibody specific for Equine TGFβ1 has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any TGFβ1 present is bound by the immobilized antibody. After washing away any unbound substances, a detection antibody specific for Equine TGFβ1 is added to the wells. Following wash to remove any unbound antibody reagent, a detection reagent is added. After intensive wash a substrate solution is added to the wells and color develops in proportion to the amount of TGFβ1 bound in the initial step. The color development is stopped and the intensity of the color is measured.

This package insert must be read in its entirety before using this product.

Storage

Store detection antibody and standard at 4°C ~ -20°C, antibody coated plate and 10 x Reagent Diluent at -20°C and the rest of the kit at 4°C. The kit can be used in 3 months.

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MATERIALS PROVIDED

Description	Quantity	Description	Quantity	Description	Quantity
Antibody Precoated Plate	1	20 x PBS	1	Substrate Solution	1
Detection Antibody	1	20 x Wash Buffer	1	Stop Solution	1
Detection Agent	1	10 x Reagent Diluent	1	DataSheet/Manual	1
Standard	3	1 N HCl	1	Neutralization Buffer	1

Bring all reagents to room temperature before use.

1 x 96-well TGFβ1 antibody coated plate -Store at -20°C upon received.

Equine TGFβ1 Detection Antibody– The lyophilized Detection Antibody should be stored at 4°C to -20°C in a manual defrost freezer for up to 6 months, if not used immediately. Centrifuge for 1 min at 5000 x g to bring down the material prior to open the vial. The vial contains sufficient Detection Antibody for a 96-well plate. Add 200 µL of 1 x Reagent Diluent to the antibody vial, vortex 20 sec and allow it to sit for 5 min prior to use. Take the entire 200 µL of detection antibody to 9.8 mL Reagent Diluent if the entire 96-well plate is used. If the partial antibody is used store the rest at -20°C until use.

Equine TGFβ1 Standard (3 vials) –The lyophilized Equine TGFβ1 Standard has a total of 3 vials. Each vial contains the standard sufficient for a 96-well plate. The non-reconstituted standard can be stored at 4°C ~ -20°C for up to 3 months if not used immediately. Centrifuge for 1 min at 5000 x g to bring down the material prior to open the tube. Add 500 µL of Reagent Diluent to the standard vial to make the high standard concentration of 2000 pg/ml. Vortex 20 sec and allow it to sit for 5 min prior to use. A seven point standard curve is generated using 2-fold serial dilutions in 1 x Reagent Diluent, vortex 20 sec for each of dilution step.

Detection Agent (50 µL) – Centrifuge for 1 min at 5000 x g to bring down the material prior to open the vial. Make 1:200 dilution in Reagent Diluent. If the entire 96-well plate is used, add all 50 µL of Detection Agent to 10 mL Reagent Diluent prior to the assay. The rest of undiluted Detection Reagent can be stored at 2 - 8°C for up to 6 months. DO NOT FREEZE.

20 x PBS, pH 7.3, 30 mL- Dilute to 1 x PBS with deionized distilled water and mix well.
20 x Wash Buffer, 25 mL- Dilute to 1 x Wash Buffer with 1 x PBS prior to use.
10 x Reagent Diluent, 3 mL –Store at -20°C. Dilute to 1 x Reagent Diluent with 1 x PBS and mix well.
Substrate Solution, 10 mL.
Stop Solution, 5 mL.
1 N HCl, 5 mL.
Neutralizaiton Buffer, 5 mL.

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Assay Procedure

1. Lift the plate cover from the top left corner and cover the wells that are not used. Vortex briefly the samples prior to the assay. Add 100 μL of activated sample (see page 5) or standard to each well and use duplicate wells for each standard or sample. Cover the 96-well plate and incubate 1 hour at room temperature.
2. Aspirate each well and wash with 1 x Wash Buffer, repeating the process two times for a total of three washes. Wash by filling each well with 1 x Wash Buffer (300 μL) using a multi-channel pipette, manifold dispenser or auto-washer. Complete removal of liquid at each step is essential for good performance. After the last wash, remove any remaining Wash Buffer by aspirating or by inverting the plate and blotting it against clean paper towels.
3. Add 100 μL of the working dilution of the Detection Antibody to each well. Cover the plate and incubate 1 hour at room temperature.
4. Repeat the aspiration/wash as in step 2.
5. Add 100 μL of the working dilution of Detection Agent to each well. Cover the plate and incubate for 20 minutes at room temperature. Avoid placing the plate in direct light.
6. Repeat the aspiration/wash as in step 2.
7. Add 100 μL of Substrate Solution to each well. Incubate for 10 minutes at room temperature. Avoid placing the plate in direct light.
8. Add 50 μL of Stop Solution to each well. Gently tap the plate to ensure thorough mixing.
9. Determine the optical density of each well immediately, using a microplate reader set to 450 nm. If wavelength correction is available, set to 540 nm or 570 nm. If wavelength correction is not available, subtract readings at 540 nm or 570 nm from the readings at 450 nm. This subtraction will correct for optical imperfections in the plate. Readings made directly at 450 nm without correction may be higher and less accurate.

Precaution and Technical Notes

1. Thorough mixing of the standard at each of dilution steps is critical to ensure a normal calibration curve. A standard curve should be generated for each set of samples assayed.
2. If TGFβ1 exceeds the upper limit of the detection, the sample needs to be diluted with PBS. The dilution factor must be used for calculation of the concentration.
3. Detection Agent contains enzyme, DO NOT mass up with Detection Antibody.
4. The Stop Solution is an acid solution, handle with caution.
5. This kit should not be used beyond the expiration date on the label.
6. A thorough and consistent wash technique is essential for proper assay performance. Wash Buffer should be dispensed forcefully and removed completely from the wells by aspiration or decanting. Remove any remaining Wash Buffer by aspiration or by inverting the plate and blotting it against clean paper towels.
7. Use a fresh reagent reservoir and pipette tips for each step.
8. It is recommended that all standards and samples be assayed in duplicate.
9. Avoid microbial contamination of reagents and buffers. This may interfere with the sensitivity of the assay.

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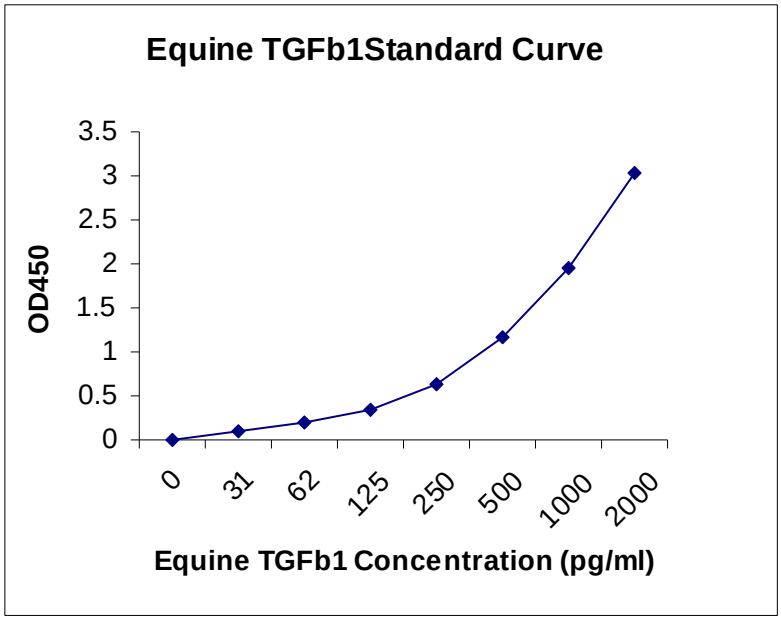
Calculation of Results

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

Create a standard curve by reducing the data 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 TGFB1 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. If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

The Standard Curve

The graph below represents typical data generated when using this Equine TGFβ1 ELISA Kit. The standard curve was calculated using a computer generated 4-PL curve-fit. For this case, a Bio-Rad iMark^{TM} Microplate Reader and a Microplate Manager 6 Software were used to generate this curve. The correlation coefficient (r^2) is 0.999-1.000.



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Specificity

The following recombinant Equine proteins prepared at 10 ng/ml were tested and exhibited no cross-reactivity or interference.
BMP1, BMP2, BMP4, HGF, IL-1β, IL-1RA, IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, IL-12, IL-13, IL-15, IFNγ, MMP-2, TLR1, TLR2, TLR3, TNF-α, TNF RI, TNF RIi, VEGF.

Calibration

This kit is calibrated against a highly purified yeast-expressed recombinant Equine TGFβ1.

Detection Range

1-2000 pg/ml

Assay Sensitivity

0.8 pg/ml

Assay Precision

Intra-Assay %CV: 5; Inter-Assay %CV: 8

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Activation of TGFβ1 in Biological Specimens

Biological specimens such as plasma need to be activated prior to TGFβ1 immunoassay.
Materials: 1 N HCl, Neutralization Buffer
Procedure

1. Add 25 µl of 1 N HCl to 50 µl of biological specimen (such as plasma) and mix well.
2. Incubate 10 min at room temperature.
3. Add 25 µl of Neutralization Buffer to neutralize the acidified sample and mix well.
4. Assay immediately. It may be a good start point if the activated sample is diluted 10-fold with 1 x Washer buffer.

Note: The activated specimens need to be diluted with 1 x Wash Buffer if its OD_{450} reading exceeds the upper limit of the standard curve and the dilution factor can be up to 20 folds depending on the TGFβ1density.

Related products

ELISA Substrate, GERC103021
ELISA Stop Solution, GERC103055
ELISA Detection Agent, GERC103044
Equine TGF-β1 standard
Equine TGF-β1 detection antibody