# Nori® 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 epithelial mesenchymal 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 Camurati Engelmann disease, a rare sclerosing bone dysplasia characterized by inappropriate presence of active TGFβ1 (8).

**References**


1. Assoian R, et al. (1983). J Biol Chem 258 (11): 7155.
2. Dunker, N. and K. Krieglstein (2000) Eur. J. Biochem.267:6982.
3. Wahl, S.M. (2006) Immunol. Rev. 213:213.
4. Chang, H. et al. (2002) Endocr. Rev. 23:787.
5. Derynck, R. et al. (1985) Nature 316:701.
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7. Miller, D.M. et al. (1992) Mol. Endocrinol. 6:694.
8. Janssens, K. et al. (2003) J. Biol. Chem. 278:7718.


## PRINCIPLE OF THE ASSAY

This ELISA kit is for quantification of TGFβ1 in equine samples. 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 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 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 at 4°C. The kit can be used in 6 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 Assay Buffer | 1 | Stop Solution | 1 |
| HRP Conjugate | 1 | Neutralization Buffer | 1 | DataSheet/Manual | 1 |
| Standard | 3 | 1 N HCl | 1 | | |

Bring all reagents to room temperature before use.

### Reagent Preparations

**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 sterile 1 x PBS 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 10.5 mL of 1 x PBS to make **working dilution of Detection Antibody** and vortex 30 sec 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) –Each of the lyophilized Equine TGFβ1 Standard 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 6 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 1 x Assay Buffer to the standard vial to make the high standard concentration of 2,000 pg/ml. **Vortex 1 min** 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 Assay Buffer, **vortex 30 sec** for each of dilution step.

**HRP Conjugate** (55 µL) – Centrifuge for 1 min at 6000 x g to bring down the material prior to open the vial. The vial contains sufficient HRP Conjugate for one 96-well plate. If the volume is less than 55 µL, add sterile 1 x PBS to reach 55 µL and vortex briefly. Make 1:200 dilution in 1 x PBS. If the entire 96-well plate is used, add 53 µL of HRP Conjugate to 10.5 mL of the PBS to make **working dilution of HRP Conjugate** and vortex 30 sec prior to the assay. The rest of undiluted HRP Conjugate can be stored at 2 - 8°C for up to 6 months. DO NOT FREEZE

**20 x PBS**, pH 7.3, 25 mL- Dilute to 1 x PBS with deionized distilled water and mix well.
**20 x Assay Buffer**, 20 mL- Dilute to 1 x Assay Buffer with 1 x PBS prior to use.
**Substrate Solution**, 10.5 mL.
**Stop Solution**, 5.5 mL.
**1 N HCl,** 2.5 mL.
**Neutralizaiton Buffer,** 2.5 mL.

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## Assay Procedure

1. Ensure **equal dispending** and remove all bubbles in the wells at each step and all procedures are conducted at room temperature. Lift the plate cover from the top left and cover the wells that are not used. **Vortex the standards and samples for 10 sec** before applying to the plate. Add 100 µL of **diluted sample** (see below) or **standard** per well and use duplicate wells for each standard or sample. Cover the 96-well plate and incubate 2 hours at room temperature. Attention: **MUST vortex standards and samples for 10 sec before pipetting to the wells!**
2. Aspirate each well and wash with **1 x Assay Buffer**, repeating the process one time for a total of two washes. Wash by filling each well with 1 x Assay 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 Assay Buffer by aspirating or by inverting the plate and blotting it against clean paper towels.
3. Add 100 µL of the **working dilution of 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 HRP Conjugate** to each well and remove air bubbles in wells. 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 and observe the color development every 1-2 mins. Incubate for up to 30 minutes (**depending on signal. Stop** the reaction when it turns to dark blue in the highest standard wells) at room temperature and remove bubbles in the wells. Over-incubation of the substrate will result in overflow of high standard and thus should be avoided. Avoid placing the plate in direct light.
8. When it gets to dark blue in the highest concentration of standard wells, add 50 µL of **Stop Solution** to each well to stop the reaction and remove bubbles in the wells. 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. It is critical to follow the procedure step by step otherwise appropriate color development may not occur as expected and make sure no air bubbles in wells before adding reagents.
2. A standard curve should be generated for each set of samples assayed. Thorough mixing of standards at each of dilution steps is critical to acquire a normal standard curve and **vortex again (10 sec) before pipetting to the 96-well plate**.
3. HRP Conjugate 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.
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.

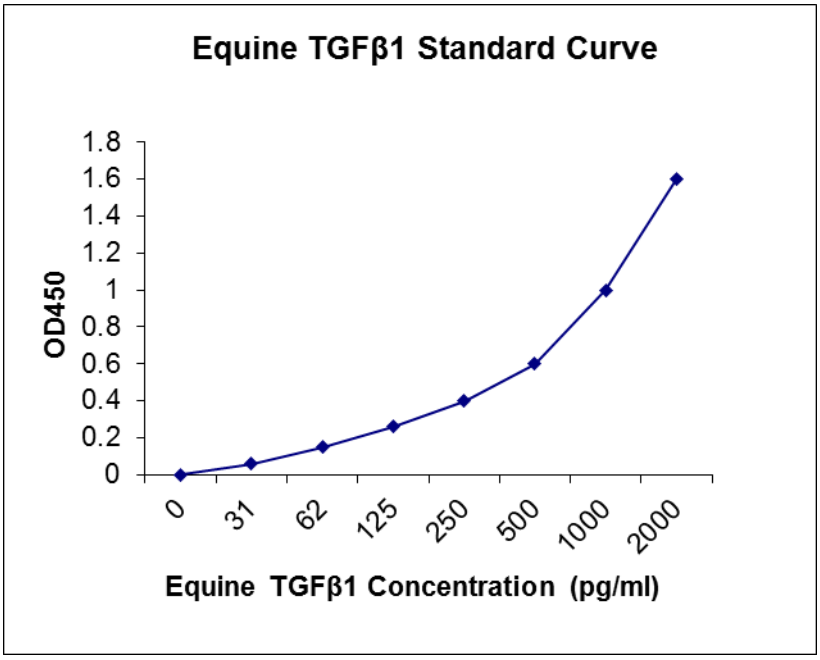
## Calculation of Results

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™ 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.

Adiponectin, ApoAI, BMP7, CRP, CCL2, FGF acidic, IGF1, HGF, HSP27, IFNγ, IL-1α, IL-1β, IL21, MMP-2, MMP-9, PDGF, PLA2G7, prolactin, serpin E1, TGFβ2, TGFβ3, TLR3, TNF-α, VEGF.

**Calibration**

This kit is calibrated against equine TGFβ1.

**Detection Range**

31-2,000 pg/ml

**Assay Sensitivity**

6 pg/ml

**Assay Precision**

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

**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 1-fold with 1 x Assay Buffer.

**Note**: The activated specimens need to be diluted with 1 x Assay Buffer if its $OD_{450}$ reading exceeds the upper limit of the standard curve and the dilution factor can be up to 10 folds depending on the TGFβ1density.

**DECLARATION**

THIS REAGENT IS FOR IN VITRO LABORATORY TESTING AND RESEARCH USE ONLY. DO NOT USE IT FOR CLINICAL DIAGNOSTICS. DO NOT USE OR INJECT IT IN HUMANS AND ANIMALS.

**FOR LABORATORY RESEARCH USE ONLY**
**NOT FOR USE IN HUMANS AND ANIMALS**

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**Troubleshooting Guide**

| Problem | Possible causes | Solution |
|---|---|---|
| Poor standard curve | • Inaccurate pipetting<br>• Insufficient vortexing<br>• $OD_{450}$ too high for the high standard point<br>• Air bubbles in wells. | • Check pipettes and ensure **equal dispensing**.<br>• Vortex 30 sec for each of standard dilution steps and **vortex again (10 sec) before pipetting to the 96-well plate**.<br>• Reduce substrate incubation time<br>• Remove air bubbles in wells by pipette tip. |
| Low signal | • Improper preparation of reagents and storage<br>• Too brief incubation times<br>• Inadequate reagent volume or improper dilution<br>• Standard defect | • Briefly spin down vials before opening. Reconstitute the powder thoroughly. Proper storage of plate and strip and detection antibody after first usage as shown in the datasheet.<br>• Ensure sufficient incubation time including substrate incubation. Increase sample incubation to 2 hours.<br>• Change a Standard vial. |
| Overflow in the standards | • Substrate incubation too long<br>• Air bubbles in wells | • Observe the color development every 1-2 mins and reduce substrate incubation time.<br>• Stop the reaction by adding 50 µl of Stop Solution when it turns to dark blue in the highest concentration of standard wells.<br>• Remove air bubbles in wells |
| Large CV | • Inaccurate pipetting and mixing<br>• Improper standard/sample dilutions.<br>• Air bubbles in wells. | • Check pipettes and ensure accurate pipetting and thorough mixing and **equal dispensing**.<br>• Use the correct dilution buffers<br>• Remove air bubbles in wells by pipette tip. |
| High background | • Reagent reservoir issue<br>• Plate is insufficiently washed and air bubbles in wells.<br>• Contaminated Assay Buffer<br>• Pipet tip contaminated | • Use a new reagent reservoir for Substrate Solution.<br>• Increase wash to 4 times before adding substrate and ensure plate washer functions normally. Remove air bubbles in wells by pipette tip.<br>• Make fresh Assay Buffer and wash thoroughly.<br>• Use new pipette tips for blank wells. |
| No signal detected | • The procedure was misconducted.<br>• Failures of spin down the contents in Detection Antibody and Standards.<br>• Failure of Substrate or HRP<br>• Samples overdiluted | • Ensure the step-by-step protocol. Spin vials of Detection antibody and Standard to complete recover the content.<br>• Mix 100 µl of Substrate with 0.5 µl HRP and deep blue color should develop in 2 min.<br>• Try a new standard vial and use positive control.<br>• Try not dilute samples |
| Low sensitivity | • Improper dilutions of standards<br>• Improper storage of the ELISA kit | • Ensure accurate and thorough dilutions of standards at each step.<br>• Store detection antibody at -20°C after reconstitution, others at 4°C. Keep substrate solution protected from light. |