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Bovine TGFβ1 ELISA Kit

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 Bovine 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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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.
6. Oklu, R. and R. Hesketh (2000) Biochem. J. 352:601.
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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 Bovine 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 Bovine 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.

MATERIALS PROVIDED

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

Bring all reagents to room temperature before use.
1 x **96-well Plate precoated with Bovine TGFβ1 capture antibody-**Store at 4 ° C upon received.

Bovine TGFβ1 Detection Antibody (60 μL) – The Detection Antibody should be stored at -20° C to -70° C in a manual defrost freezer for up to 6 months, if not used immediately. Spin to bring down the material prior to open the vial. The vial contains sufficient Detection Antibody for a 96-well plate. Vortex briefly and allow it to sit for a minimum of 15 min prior to use. Take the entire 60 µL of detection antibody to 10 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.

Bovine TGFβ1 Standard (3 vials) – Bovine TGFβ1 Standard has a total of 3 vials. Each vial contains 12 μL of the standard sufficient for a 96-well plate. The undiluted standard can be stored at -20° C for up to 2 months if not used immediately. Spin to bring down the material prior to open the tube. The vial contains sufficient Bovine TGFB1 standard protein for three 96- well plates. Add 12 µL of the standard to 488 µL of Reagent Diluent to make the high standard concentration of 8000 pg /ml. Vortex briefly and allow it to sit for a minimum of 15 min prior to use. A seven point standard curve is generated using 2-fold serial dilutions in Reagent Diluent, vortex briefly for each of dilution step. Store the rest of the standard at -20° C.

Detection Agent (50 μL) – 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.
10 x Wash Buffer, 40 mL- Dilute to 1 x Wash Buffer with 1 x PBS prior to use.
10 x Reagent Diluent, 3 mL –dilute to 1 x Reagent Diluent with 1 x PBS and mix well.
Substrate Solution, 12 mL.
Stop Solution, 6 mL.

2016@Advanced BioReagents Systems, Hayward, CA 94545
Tel:650 458-0155 info@abioreagents.com order@abioreagents.com www.abioreagents.com Cat#ABS3019

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Assay Procedure

1. Lift the plate cover. Vortex briefly the samples prior to the assay. Add 100 μL of sample or less to each well, and in each well add PBS to a total volume of 100 μL if the sample is less than 100 μL. Add 100 μL of standards per 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 10-20 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. 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.
2. Detection Agent contains enzyme, DO NOT mass up with Detection Antibody.
3. The Stop Solution is an acid solution, handle with caution.
4. A standard curve should be generated for each set of samples assayed.
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.
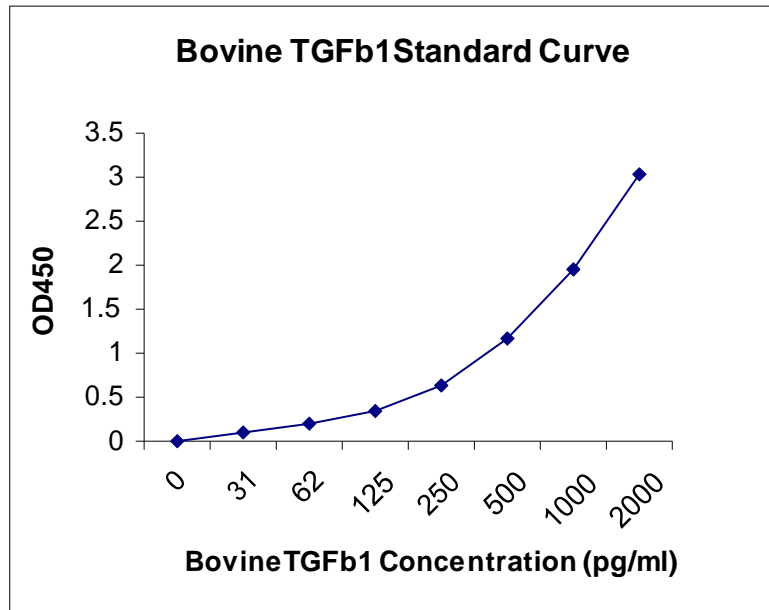
Calculation of Results

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 Bovine 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.

2016@Advanced BioReagents Systems, Hayward, CA 94545
Tel:650 458-0155 info@abioreagents.com order@abioreagents.com www.abioreagents.com Cat#ABS3019

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Specificity

The following recombinant Bovine proteins prepared at 10 ng/ml were tested and exhibited no cross-reactivity or interference.

IL-1β, IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, IL-15, IFNγ, TLR1, TLR2, TNF-α.

Calibration

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

Detection Range

1-8000 pg/ml

Assay Sensitivity

0.6 pg/ml

Assay Precision

Intra-Assay %CV: 6; Inter-Assay %CV: 11

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