OriGene Technologies Inc.
9620 Medical Center Drive, Suite 200, Rockville, MD 20850
Phone: 1.888.267.4436 Fax: 301-340-9254
Email: techsupport@origene.com Web: www.origene.com

Mouse TGF Beta 3 ELISA Kit

Catalog Number: EA100647

Assay Principle

The OriGene Mouse Tgfb3 Pre-Coated ELISA (Enzyme-Linked Immunosorbent Assay) kit is a solid phase immunoassay specially designed to measure Mouse Tgfb3 with a 96-well strip plate that is pre-coated with antibody specific for TGFB3. The detection antibody is a biotinylated antibody specific for TGFB3. The capture antibody is monoclonal antibody from rat, the detection antibody is biotinylated polyclonal antibody from goat. The kit contains recombinant Mouse Tgfb3 with immunogen: Expression system for standard: NSO, Immunogen sequence: A301-S412. The kit is analytically validated with ready to use reagents.

To measure Mouse Tgfb3, add standards and samples to the wells, then add the biotinylated detection antibody. Wash the wells with PBS or TBS buffer, and add Avidin-Biotin-Peroxidase Complex (ABC-HRP). Wash away the unbounded ABC-HRP with PBS or TBS buffer and add TMB. TMB is substrate to HRP and will be catalyzed to produce a blue color product, which changes into yellow after adding acidic stop solution. The density of the yellow product is linearly proportional to Mouse Tgfb3 in the sample. Read the density of the yellow product in each well using a plate reader, and benchmark the sample wells' readings against the standard curve to determine the concentration of Mouse Tgfb3 in the sample.

Overview

Product Name	Mouse TGFB3 ELISA Kit
Reactive Species	Mouse
Size	96wells/kit, with removable strips.
Description	Mouse TGFB3 ELISA Kit (96 Tests). Quantitate Mouse Tgfb3 in cell culture supernatants, serum and plasma (EDTA) und urine. Sensitivity: 10 pg/ml
Sensitivity	< 10pg/ml *The sensitivity or the minimum detectable dose (MDD) is the lower limit of target protein that can be detected by the kit. It is determined by adding two standard deviations to the mean O.D. value of twenty (20) blank wells and calculating the corresponding concentration.
Detection Range	31.2 pg/ml – 2000 pg/ml
Storage Instructions	Store at 4°C for 6 months, at -20°C for 12 months. Avoid multiple freeze-thaw cycles (Shipped with wet ice.)
Uniprot ID	P17125

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Technical Details

Capture/Detection Antibodies	*The capture antibody is monoclonal antibody from rat, the detection antibody is biotinylated polyclonal antibody from goat.*
Specificity	*Natural and recombinant Mouse Tgfb3*
Immunogen	*Expression system for standard: NSO, Immunogen sequence: A301-S412*
Cross Reactivity	This kit is for the detection of Mouse Tgfb3. No significant cross-reactivity or interference between TGFB3 and its analogs was observed. This claim is limited by existing techniques therefore cross-reactivity may exist with untested analogs.

Preparation before Assay

Please read the following instructions before starting the experiment.
1. Read this manual in its entirety in order to minimize the chance of error.
2. Confirm that you have the appropriate non-supplied equipment available.
3. Confirm that the species, target antigen, and sensitivity of this kit are appropriate for your intended application.
4. Confirm that your samples have been prepared appropriately based upon recommendations (see Sample Preparation) and that you have sufficient sample volume for use in the assay.
5. When first using a kit, appropriate validation steps should be taken before using valuable samples. Confirm that the kit adequately detects the target antigen in your intended sample type(s) by running control samples.
6. If the concentration of target antigen within your samples is unknown, a preliminary experiment should be run using a control sample to determine the optimal sample dilution (see Sample Preparation).
7. To inspect the validity of experiment operation and the appropriateness of sample dilution proportion, a pilot experiment using standards and a small number of samples is recommended.
8. Before using the kit, spin tubes to bring down all components to the bottom of the tubes.
9. Don’t let the 96-well plate dry out since this will inactivate active components on the plate.
10. Don’t reuse tips and tubes to avoid cross-contamination.
11. Avoid using the reagents from different batches together.
12. The kit should not be used beyond the expiration date on the kit label. Any variation in diluent, operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding. Variations in sample collection, processing, and storage may cause sample value differences.

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Kit Components/Materials Provided

Description	Quantity	Volume	
Anti-Mouse Tgfb3 Pre-coated 96-well strip microplate	1	12 strips of 8 wells	Storage of opened/reconstituted material
Mouse Tgfb3 Standard	2	10 ng/tube	Return unused wells to the foil pouch. Reseal along the entire edge of the zip-seal. May be stored for up to 1 month at 4°C provided this is within the expiration date of the kit.
Mouse Tgfb3 Biotinylated antibody (100x)	1	100 µl	Discard the TGFB3 stock solution after 12 hours at 4°C. May be stored at -20°C for 48 hours.
Avidin-Biotin-Peroxidase Complex (100x)	1	100 µl	May be stored for up to 1 month at 4°C provided this is within the expiration date of the kit.
Sample Diluent	1	30ml	
Antibody Diluent	1	12ml	
Avidin-Biotin-Peroxidase Diluent	1	12ml	
Color Developing Reagent (TMB)	1	10ml	
Stop Solution	1	10ml	
Wash Buffer (25x)	1	20ml	
Plate Sealers	4	Piece	

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Required Materials That Are Not Supplied

Microplate Reader capable of reading absorbance at 450nm.
Automated plate washer (optional)
Pipettes and pipette tips capable of precisely dispensing 0.5 µl through 1 ml volumes of aqueous solutions.
Multichannel pipettes are recommended for large amount of samples.
Deionized or distilled water.
500ml graduated cylinders.
Test tubes for dilution.

Mouse TGFB3 ELISA Kit (EA100647) Standard Curve Example

Highest O.D. value might be higher or lower than in the example. The experiment result is statistically significant if the highest O.D. value is no less than 1.0.

Concentration (pg/ml)	0	31.25	62.50	125	250	500	1000	2000
O.D.	0.025	0.065	0.105	0.175	0.311	0.657	1.054	1.877

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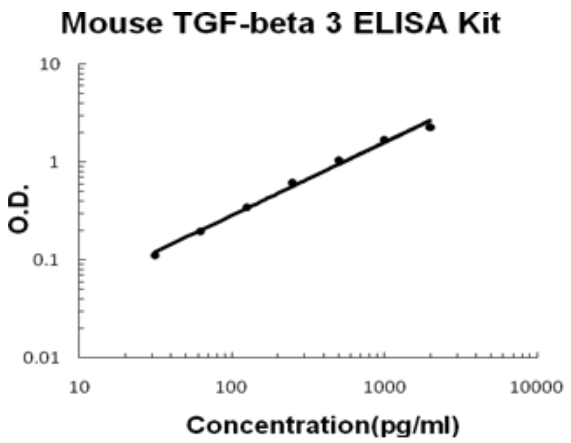
Mouse Tgfb3 ELISA Kit standard curve



A standard curve is provided for demonstration only. A standard curve should be generated for each set of samples assayed.

Intra/Inter Assay Variability

OriGene spend great efforts in documenting lot to lot variability and make sure our assay kits produce robust data that are reproducible.

Intra-Assay Precision (Precision within an assay): *Three samples of known concentration were tested on one plate to assess intra-assay precision.*

Inter-Assay Precision (Precision across assays):*Three samples of known concentration were tested in separate assays to assess inter-assay precision.*

	Intra-Assay Precision			*Inter-Assay Precision*		
Sample	*1*	*2*	*3*	*1*	*2*	*3*
n	*16*	*16*	*16*	*24*	*24*	*24*
Mean(pg/ml)	*58*	*278*	*1013*	*63*	*286*	*988*
Standard deviation	*2.61*	*15.01*	*45.58*	*3.59*	*15.70*	*57.30*
CV(%)	*4.5 %*	*5.4 %*	*4.5 %*	*5.7 %*	*5.7 %*	*5.8 %*

Reproducibility

To assay reproducibility, three samples with differing target protein concentrations were assayed using four different lots.

Lots	*Lot1 (pg/ml)*	*Lot2 (pg/ml)*	*Lot3 (pg/ml)*	*Lot4 (pg/ml)*	*Mean (pg/ml)*	*Standard Deviation*	*CV (%)*
Sample 1	*58*	*64*	*58*	*53*	*58*	*3.89*	*6.7 %*
Sample 2	*278*	*301*	*266*	*300*	*286*	*14.87*	*5.1 %*
Sample 3	*1013*	*982*	*950*	*928*	*968*	*32.18*	*3.3 %*

**number of samples for each test n=16.*

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Preparation Before The Experiment

Item	Preparation
All reagents	Bring all reagents to room temperature (18-25°C) prior to use. Please DO NOT equilibrate unused plate well strips to room temperature. They should be sealed and stored in the original packaging. The assay can also be done at room temperature however we recommend doing it at 37°C for best consistency with our QC results. Also, the TMB incubation time estimate (15-25 min) is based on incubation at 37°C.
Wash buffer	Prepare 500 ml of Working Wash Buffer by diluting the supplied 20 ml of Wash Buffer (25 x) with 480 ml of deionized or distilled water. If crystals have formed in the concentrate, warm to room temperature and mix it gently until crystals have completely dissolved.
Biotinylated Anti-Mouse Tgfb3 antibody	It is recommended to prepare this reagent immediately prior to use by diluting the Mouse Tgfb3 Biotinylated antibody (100x) 1:100 with Antibody Diluent. Prepare 100 µl by adding 1 µl of Biotinylated antibody (100x) to 99 µl of Antibody Diluent for each well. Mix gently and thoroughly and use within 2 hours of generation.
Avidin-Biotin-Peroxidase Complex	It is recommended to prepare this reagent immediately prior to use by diluting the Avidin-Biotin-Peroxidase Complex (100x) 1:100 with Avidin-Biotin-Peroxidase Diluent. Prepare 100 µl by adding 1 µl of Avidin-Biotin-Peroxidase Complex (100x) to 99 µl of Avidin-Biotin-Peroxidase Diluent for each well. Mix gently and thoroughly and use within 2 hours of generation.
Mouse Tgfb3 Standard	It is recommended that the standards be prepared no more than 2 hours prior to performing the experiment. Use one 10 ng of lyophilized Mouse Tgfb3 standard for each experiment. Gently spin the vial prior to use. Reconstitute the standard to a stock concentration of 10 ng/ml using 1ml of sample diluent. Allow the standard to sit for a minimum of 10 minutes with gentle agitation prior to making dilutions.
Microplate	The included microplate is coated with capture antibodies and ready-to-use. It does not require additional washing or blocking. The unused well strips should be sealed and stored in the original packaging.

Dilution of Mouse Tgfb3 Standard

1. Number tubes 1-8. Final Concentrations to be Tube # 1 –2000 pg/ml, #2 –1000 pg/ml, #3 – 500 pg/ml, #4 – 250 pg/ml, #5 – 125 pg/ml, #6 – 62.50 pg/ml, #7 – 31.25pg/ml, #8 – 0.0 (Blank).
2. For standard #1, add 200µl of the reconstituted standard stock solution of 10 ng/ml and 800 µl of sample diluten to tube #1 for a final colume of 1000 µl.
3. Add 300 µl of sample diluent to tubes # 2-7.
4. To generate standard #2, add 300 µl of standard #1 from tube #1 to tube #2 for a final volume of 600 µl. Mix thoroughly.
5. To generate standard #3, add 300 µl of standard #2 from tube #2 to tube #3 for a final volume of 600 µl. Mix thoroughly.
6. Continue the serial dilution for tube #4-7.
7. These sample collection instructions and storage conditions are intended as a general guideline and the sample stability has not been evaluated.

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Sample Preparation and Storage

Sample Type	Procedure
Cell culture supernatants	Clear sample of particulates by centrifugation, assay immediately or store samples at -20°C.
Serum	Use a serum separator tube (SST) and allow serum to clot at room temperature for about four hours. Then, centrifuge for 15 min at approximately 1,000 x g. assay immediately or store samples at -20°C.
Plasma	Collect plasma using EDTA as an anticoagulant. Centrifuge for 15 min at approximately 1,000 x g. Assay immediately or store samples at -20°C. *Note: it is important to not use anticoagulants other than the ones described above to treat plasma for other anticoagulants could block the antibody binding site.
Urine	Collect the first urine of the day, micturate directly into sterile container. Remove impurities by centrifugation, assay immediately or aliquot and store samples at -20°C.

Note: To detect Tgfb3 in samples, you need to activate Tgfb3 in samples prior to the assay!

Activate the Sample:

Tgfb3 is mostly contained as inactive form in samples, please activate it before assay. Do not activate recombinant Tgfb3.

Solution A: 1N HCl: add 8.33 ml of 12N HCl into 91.67 ml of H20.
Solution B: 1.2N NaOH/0.5M HEPES: add 12 ml of 10N NaOH and 11.9 g HEPES into 75ml of H2O; add H2O to adjust volume to 100 ml.

Cell culture supernatants, urine: add activating reagent pro rata, i.e. add 20 µl of Solution A into 100 µl of sample; 10 min later add 20 µl of Solution B, PH 7.0-7.6.

Serum, plasma (EDTA). Add activating reagent pro rata, i.e. add 20 µl of Solution A into 40 µl of sample; 10 min later add 20 µl of Solution B, PH 7.0-7.6.

It is unnecessary to activate recombinant Tgfb3.

Sample was diluted partly after adding activating reagent, so please pay attention to this when calculating target protein concentration.

Sample collection notes

1. OriGene recommends that samples are used immediately upon preparation.
2. Avoid repeated freeze/thaw cycles for all samples.
3. In the event that a sample type not listed above is intended to be used with the kit, it is recommended that the customer conduct validation experiments in order to be confident in the results.
4. Due to chemical interference, the use of tissue or cell extraction samples prepared by chemical lysis buffers may result in inaccurate results.
5. Due to factors including cell viability, cell number, or sampling time, samples from cell culture supernatant may not be detected by the kit.

Sample dilution Guideline

The user needs to estimate the concentration of the target protein in the sample and use an appropriate dilution factor so that the diluted target protein concentration falls in the range of O.D. values of the standard curve. Dilute the sample using provided diluent buffer. Pilot tests using a dilution series of each sample type are necessary. The sample must be mixed thoroughly with Sample Diluent.

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Assay protocol

It is recommended that all reagents and materials be equilibrated to 37°C/room temperature prior to the experiment (see Preparation Before The Experiment if you have missed this information).

1. *Prepare all reagents and working standards as directed previously.*
2. *Remove excess microplate strips from the plate frame and seal and store them in the original packaging.*
3. *Add 100 µl of the standard, samples, or control per well. Add 100 µl of the sample diluent buffer into the control well (Zero well). At least two replicates of each standard, sample, or control is recommended.*
4. *Cover with the plate sealer provided and incubate for 120 minutes at RT (or 90 min. at 37 °C).*
5. *Remove the cover and discard the liquid in the wells into an appropriate waste receptacle. Invert the plate on the benchtop onto a paper towel and tap the plate to gently blot any remaining liquid. It is recommended that the wells are not allowed to completely dry at any time.*
6. *Add 100 µl of the prepared 1x Biotinylated Anti-Mouse Tgfb3 antibody to each well.*
7. *Cover with plate sealer and incubate for 90 minutes at RT (or 60 minutes at 37°C).*
8. *Wash the plate 3 times with the 1x wash buffer.*
   a. *Discard the liquid in the wells into an appropriate waste receptacle. Then, invert the plate on the benchtop onto a paper towel and tap the plate to gently blot any remaining liquid. It is recommended that the wells are not allowed to completely dry at any time.*
   b. *Add 300 µl of the 1x wash buffer to each assay well. (For cleaner background incubate for 60 seconds between each wash).*
   c. *Repeat steps a-b 2 additional times.*
9. *Add 100 µl of the prepared 1x Avidin-Biotin-Peroxidase Complex into each well. Cover with the plate sealer provided and incubate for 40 minutes at RT (or 30 minutes at 37°C).*
10. *Wash the plate 5 times with the 1x wash buffer.*
    a. *Discard the liquid in the wells into an appropriate waste receptacle. Then, invert the plate on the benchtop onto a paper towel and tap the plate to gently blot any remaining liquid. It is recommended that the wells are not allowed to completely dry at any time.*
    b. *Add 300 µl of the 1x wash buffer to each assay well. (For cleaner background incubate for 60 seconds between each wash).*
    c. *Repeat steps a-b 4 additional times.*
11. *Add 90 µl of Color Developing Reagent to each well. Cover with the plate sealer provided and incubate in the dark for 30 minutes at RT (or 15-25 minutes at 37°C). (The optimal incubation time must be empirically determined. A guideline to look for is blue shading the top four standard wells, while the remaining standards remain clear.)*
12. *Add 100 µl of Stop Solution to each well. The color should immediately change to yellow.*
13. *Within 30 minutes of stopping the reaction, the O.D. absorbance should be read with a microplate reader at 450nm.*

Data Analysis

Average the duplicate readings for each standard, sample, and control. Subtract the average zero standard O.D. reading.

It is recommended that a standard curve be created using computer software to generate a four parameter logistic (4-PL) curve-fit. A free program capable of generating a four parameter logistic (4-PL) curve-fit can be found online at: www.myassays.com/four-parameter-logistic-curve.assay.

Alternatively, plot the mean absorbance for each standard against the concentration. The measured concentration in the sample can be interpolated by using linear regression of each average relative OD against the standard curve generated using curve fitting software. This will generate an adequate but less precise fit of the data.

For diluted samples, the concentration reading from the standard curve must be multiplied by the dilution factor.

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Background on TGFB3

Transforming growth factor beta 3 (TGF-beta 3) is a type of protein, known as a cytokine, which is involved in cell differentiation, embryogenesis and development. It belongs to a large family of cytokines called the Transforming growth factor beta superfamily. TGF-beta 3 is believed to regulate molecules involved in cellular adhesion and extracellular matrix (ECM) formation during the process of palate development. Without TGF-beta 3, mammals develop a deformity known as acleft palate. This is caused by failure of epithelial cells in both sides of the developing palate to fuse. TGF-beta 3 also plays an essential role in controlling the development of lungs in mammals, by also regulating cell adhesion and ECM formation in this tissue, and controls wound healing by regulating the movements of epidermal and dermal cells in injured skin. TGF-beta 3 activated Lef1 in the absence of beta-catenin (CTNNB1) via nuclear phospho-Smad2 and Smad4.

Reference

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3. *Taya Y, O'Kane S, Ferguson M (1999). "Pathogenesis of cleft palate in TGF-beta3 knockout mice". Development 126(17): 3869–79.*