Mouse Tenascin (TNC) ELISA Kit

Item NO.	Product Name
KTE70138	Mouse Tenascin (TNC) ELISA Kit

ATTENTION

For laboratory research use only. Not for clinical or diagnostic use

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INTRODUCTION

Assay principle

Mouse Tenascin (TNC) ELISA Kit employs a two-site sandwich ELISA to quantitate TNC in samples. An antibody specific for Mouse TNC has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any TNC present is bound by the immobilized antibody. After removing any unbound substances, HRP-Conjugated TNC detection antibody is added to the wells. Following a wash to remove any unbound HRP reagent, a Chromogen solution is added to the wells and color develops in proportion to the amount of TNC bound in the initial step. The color development is stopped and the intensity of the color is measured.

Characteristics

- This Kit allows for the determination of TNC concentrations in Mouse serum, cell culture supernates and other biological fluids.
- Detection range: 50 pg/ml - 800 pg/ml.
- The minimum detectable dose (MDD) of Mouse TNC is typically less than 5 pg/ml.
- Four samples of known concentration were tested twenty times on one plate to assess intra-assay precision. The CV (%)＜9%.
- Three samples of known concentration were tested in twenty separate assays to assess inter-assay precision. Assays were performed by at least three technicians using two lots of components. The CV (%)＜11%.
- To assess linearity of the assay, samples containing and/or spiked with high concentrations of Mouse TNC were diluted with the appropriate calibrator diluent to produce samples with values within the dynamic range of the assay. Linear regression analysis of samples versus the expected concentration yielded a correlation coefficient of 0.99.
- Mouse Tenascin (TNC) ELISA Kit has high sensitivity and excellent specificity for detection of Mouse TNC. No significant cross-reactivity or interference between Mouse TNC and analogues was observed.

PRODUCT INFORMATION

Materials supplied & Storage conditions

Store kit reagents at 2-8 ℃ for 12 months. Immediately after use remaining reagents should be returned to cold storage at 4 ℃.

components	48T	

ASSAY PROTOCOL

Sample collection & storage

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Tissue homogenates - For general information, hemolysis blood may affect the result, so you should rinse the tissues with ice-cold PBS (0.01M, pH=7.4) to remove excess blood thoroughly. Tissue pieces should be weighed and then minced to small pieces which will be homogenized in PBS with a glass homogenizer on ice. (The volume depends on the weight of the tissue, 9mL PBS would be appropriate to 1 gram tissue pieces. Some protease inhibitor is recommended to add into the PBS.) To further break the cells, you can sonicate the suspension with an ultrasonic cell disrupter or subject it to freeze-thaw cycles. The homogenates are then centrifugated for 5 minutes at 5000×g to collect the supernate.

Cell culture supernatants and other biological fluids - Centrifuge samples for 20 minutes at 1000×g. Remove particulates and assay immediately or store samples in aliquot at -20 ℃ or -80 ℃ for later use. Avoid repeated freeze/thaw cycles.

Serum - Use a serum separator tube and allow samples to clot for two hours at room temperature or overnight at 4 ℃ before centrifugation for 20 minutes at approximately 1000×g. Assay freshly prepared serum immediately or store samples in aliquot at -20 ℃ or -80 ℃ for later use. Avoid repeated freeze/thaw cycles.

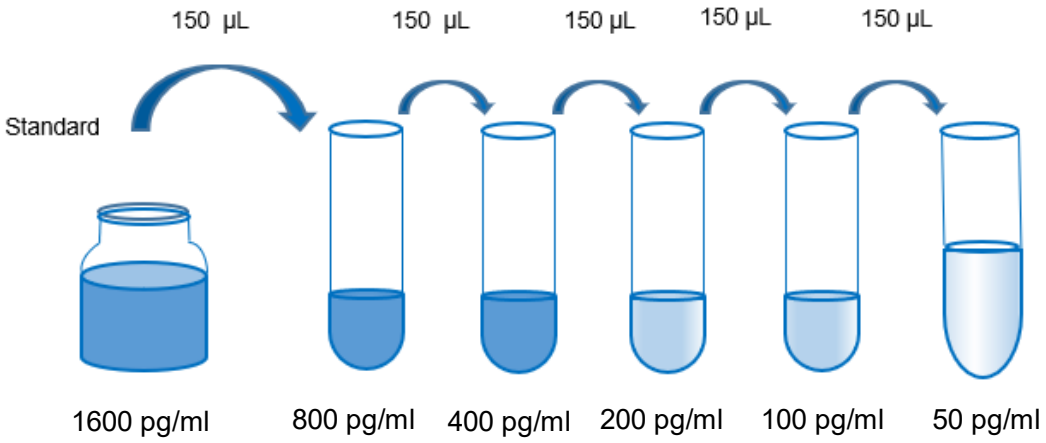
Plasma - Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge samples for 15 minutes at 1000×g at 2-8 ℃ within 30 minutes of collection. Remove plasma and assay immediately or store samples in aliquot at -20 ℃ or -80 ℃ for later use. Avoid repeated freeze/thaw cycles.
Note: Samples should be centrifugated adequately and no hemolysis or granule was allowed.

Reagent preparation

Bring all reagents to room temperature before use. If crystals were formed in the Buffer Concentrates, warm them gently until they completely dissolved.

Wash buffer - Dilute with Distilled or deionized water 1:20 (48T) /1:30 (96T).

Standard - Pipette 150 µL of Standard Diluent into each tube. Use the stock solution to produce a 2-fold dilution series (below). Mix each tube thoroughly before the next transfer. The undiluted Standard serves as the high standard.



Note: If samples generate values higher than the highest standard, please dilute the samples with Sample Diluent and repeat the assay.

Assay procedure

1. Prepare all reagents before starting assay procedure. It is recommended that all Standards and Samples be added in duplicate to the microplate.
2. Add standard: Set Standard wells, testing sample wells. Add diluted standard 50 µL to standard well.
3. Add Sample: Add sample diluent 40 µL to testing sample well. Then add sample 10 µL to testing sample well, Blank well doesn't add anything.
4. Cover with a plate cover and incubate for 45 minutes at 37 ℃.
5. Aspirate each well and wash, repeating the process four times for a total of five washes, 1-3 minutes per time. Wash by filling each well with Wash buffer (250 µL) using a squirt bottle, manifold dispenser or autowasher. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
6. Add HRP-Conjugated detection antibody 50 µL to each well, except blank well.
7. Cover with plate cover. Incubate for 30 minutes at 37 ℃.
8. Repeat the aspiration/wash process for five times as in step 5.
9. Add chromogen solution A 50 µL and chromogen solution B 50 µL to each well. Gently mix and incubate for 15 minutes at 37℃. Protect from light.
10. Add 50 µL Stop Solution to each well. The color in the wells should change from blue to yellow. If the color in the wells is green or the color change does not appear uniform, gently tap the plate to ensure thorough mixing.
11. Read the Optical Density (O.D.) at 450 nm using a microtiter plate reader within 15 minutes.

DATA ANALYSIS

Calculation of results

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

PRECAUTIONS

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