Code No. 27767

Tenascin-C Large (FNⅢ-B) Assay Kit - IBL

INTRODUCTION

Tenascin-C is an extracellular matrix (ECM) glycoprotein that is composed of 210-400 kDa subunits consisting of four domains. One subunit has a TA domain at the N-terminal end, then an epidermal growth factor-like sequence domain (EGF-like domain), a fibronectin type Ⅲ (FNⅢ) repeat domain, and a fibrinogen-like domain at the C-terminal end. There is an alternatively spliced domain in the FNⅢ domain, and it generates some types of variants of Tenascin-C. The subunits form a trimer by twisting at the N-terminal coiled domain and form a hexamer by a disulfide bond, in tissue. While low molecular weight variants of Tenascin-C are present in normal tissue, it is said that high molecular variants of Tenascen-C is expressed in various diseased tissue including cancer.
This kit can detect the Tenascin-C high molecular weight variant including the subunit in which FNⅢ-B domain specifically, by using two different kinds of specific antibodies.

■ "Large" in the product name means "high molecular weight variant"

IBL Tenascin-C Product Lines:

Code No.	Name	Volume
27767	**Tenascin-C Large (FNⅢ-B) Assay Kit -IBL**	**96 Well**
27751	Human Tenascin-C Large (FNⅢ-C) Assay Kit -IBL	96 Well

PRINCIPLE

This kit is a solid phase sandwich ELISA using 2 kinds of highly specific antibodies. Tetra Methyl Benzidine (TMB) is used as a coloring agent (Chromogen). The strength of coloring is proportional to the quantity of Tenascin-C High Molecular Weight Variants including FNⅢ-B domain.

Coating Antibody : Anti-Tenascin-C (4C8MS) Mouse IgG MoAb Affinty Purify
: specific to FNⅢ-B domain
Labeled Antibody : HRP conjugated Anti-Tenascin-C (4F10TT) Mouse IgG Fab' Affinty Purify
: react with EGF-like domain

MEASUREMENT RANGE

0.20 ～ 12.5 ng/mL

INTENDED USE

- This kit can detect Tenascin-C high molecular weight variants including FNⅢ-B domain in serum of human, mouse and rat.
- This kit can detect Tenascin-C high molecular weight variants including FNⅢ-B domain in EDTA plasma of human, mouse and rat.
- Serum or plasma samples are recommended to be diluted to 400～1,600-fold by EIA buffer or PBS in advance.
- This kit can detect Tenascin-C high molecular weight variants including FNⅢ-B domain in cell culture supernatant. Since this kit also cross-react with Tenascen-C in FCS, it is recommended to use serum-free culture medium. In the case of using FCS by necessity, set the medium control and substract its value from the measurement value.

KIT COMPONENT

1	Precoated plate : Anti-Tenascen-C (4C8MS) Mouse IgG MoAbAffinity Purify	96Well x 1
2	Labeled antibody Conc. : (30X) HRP conjugated Anti- Tenascin-C (4F10TT) Mouse IgG Fab' Affinity Purify	0.4mL x 1
3	Standard : Purified Human Tenascin-C	0.5mL x 2
4	EIA buffer*	30mL x 1
5	Solution for Labeled antibody*	12mL x 1
6	Chromogen : TMB solution	15mL x 1
7	Stop solution*	12mL x 1
8	Wash buffer Conc.*	50mL x 1

OPERATION MANUAL

1. Materials needed but not supplied

- Plate reader (450nm)
- Micropipette and tip
- Graduated cylinder and beaker
- Deionized water
- Refrigerator (as 4°C)
- Graph paper (log/log)
- Paper towel
- Tube for dilution of Standard
- Incubator (37°C±1°C)
- PBS (for sample dilution)
- Washing bottle for precoated plate
- Disposable test tube for "2, Labeled antibody Conc." and "6, Chromogen"

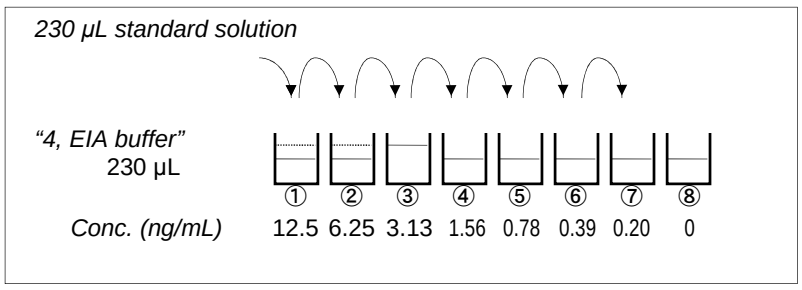
2. Preparation

1) Preparation of wash buffer
"8, Wash buffer Conc." is a concentrated (40X) buffer. Adjust the temperature of "8, Washing buffer Conc." to room temperature and then, mix it gently and completely before use. Dilute 50mL of "8, Wash buffer Conc." with 1,950mL of deionized water and mix it. This is the wash buffer for use. This prepared wash buffer shall be stored in refrigerator and used within 2 weeks after dilution.
2) Preparation of Labeled antibody
"2, Labeled antibody Conc." is a concentrated (30X). Dilute "2, Labeled antibody Conc." with "5, Solution for Labeled antibody" in 30 times according to required quantity into a disposable test tube. Use this resulting solution as Labeled antibody.
Example)
In case you use one slit (8 well), the required quantity of Labeled antibody is 800 µL. (Dilute 30 µL of "2, Labeled antibody Conc." with 870 µL of "5, Solution for Labeled antibody" and mix it. And use the resulting solution by 100 µL in each well.)
<u>This operation should be done just before the application of Labeled antibody.</u>
The remaining "2, Labeled antibody Conc." should be stored at 4°C in firmly sealed vial.
3) Preparation of Standard
Put just <u>0.5 mL</u> of deionized water into the vial of "3, Standard" and mix it gently and completely. This solution is 25 ng/mL Tenascin-C standard.
4) Dilution of Standard
Prepare 8 tubes for dilution of "3, Standard". Put 230 µL each of "4, EIA buffer" into the tube.
Specify the following concentration of each tube."

Tube-1	12.5 ng/mL
Tube-2	6.25 ng/mL
Tube-3	3.13 ng/mL
Tube-4	1.56 ng/mL
Tube-5	0.78 ng/mL
Tube-6	0.39 ng/mL
Tube-7	0.20 ng/mL
Tube-8	0 ng/mL (Test Sample Blank)

Put 230 µL of Standard solution into tube–1 and mix it gently. Then, put 230 µL of tube-1 mixture into tube-2. Dilute two times standard solution in series to set up 7 points of diluted standard between 12.5 ng/mL and 0.20 ng/mL. Tube-8 is the test sample blank as 0 ng/mL.

See following picture.



5) Dilution of test sample
Test sample may be diluted with "4, EIA buffer" or PBS as necessary.
If the concentration of Tenascin-C in samples may not be estimated in advance, the pre-assay with several different dilutions will be recommended to determine the proper dilution of samples.

3. Measurement procedure

All reagents shall be brought to room temperature approximately 30 minutes before use. Then mix it gently and completely before use. Confirm no change in quality of the reagents. Standard curve shall be prepared simultaneously with the measurement of test samples.

Reagents	Test Sample	Standard	Test Sample Blank	Reagent Blank
	Test sample 100 µL	Diluted standard (Tube 1~7) 100 µL	EIA buffer (Tube-8) 100 µL	EIA buffer 100 µL
Incubation for 60 minutes at 37°C with plate lid				
4 times (wash buffer more than 350 µL)*				
Labeled Antibody	100 µL	100 µL	100 µL	-
Incubation for 30 minutes at 4°C with plate lid				
5 times (wash buffer more than 350 µL)*				
Chromogen	100 µL	100 µL	100 µL	100 µL
Incubation for 30 minutes at room temperature (shielded)				
Stop solution	100 µL	100 µL	100 µL	100 µL
Read the plate at 450nm against a Reagent Blank within 30 minutes after addition of Stop solution.				

1) Determine wells for reagent blank. Put 100 µL each of "4, EIA buffer" into the wells.
2) Determine wells for test sample blank, test sample and diluted standard. Then, put 100 µL each of test sample blank (tube-8), test sample and dilutions of standard (tube-1~7) into the appropriate wells.
3) Incubate the precoated plate for 60 minutes at 37°C after covering it with plate lid.
4) Wash the plate with the prepared wash buffer and remove all liquid.*
5) Pipette 100 µL of labeled antibody solution into the wells of test samples, diluted standard and test sample blank.
6) Incubate the precoated plate for 30 minutes at 4°C after covering it with plate lid.
7) Wash the plate with the prepared wash buffer and remove all liquid.*
8) "6, Chromogen" should be taken the required quantity into a disposable test tube. Then, pipette 100 µL from the test tube into the wells. Please avoid returning the rest of test tube into "6, Chromogen" bottle due to avoid causing of contamination.
9) Incubate the precoated plate for 30 minutes at room temperature in the dark. The liquid will turn blue by the addition of "6, Chromogen".
10) Pipette 100 µL of "7, Stop solution" into the wells. Mix the liquid by tapping the side of precoated plate. The liquid will turn yellow by the addition of "7, Stop solution".
11) Remove any dirt or drop of water on the bottom of the precoated plate and confirm there is no bubble on the surface of the liquid. Then, run the plate reader and conduct measurement at 450nm against a Reagent Blank.
The measurement shall be done within 30minutes after the addition of "7, Stop solution".

SPECIAL ATTENTION

1) Test samples should be measured soon after the collection. For storage of test samples, store them frozen and do not repeat freeze/thaw cycles. Thaw the test samples at low temperature and mix them completely before measurement.
2) Test samples should be diluted with "4, EIA buffer" or PBS, if the need arises.
3) Duplicate measurement of test samples and standard is recommended.
4) Use test samples in neutral pH range. The contaminations of organic solvent may affect the measurement.

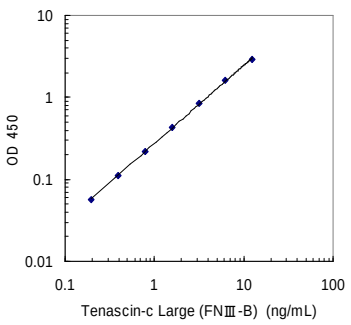
5) Use only wash buffer contained in this kit for washing the precoated plate. Insufficient washing may lead to the failure in measurement.
6) Remove the wash buffer completely by tapping the precoated plate on paper towel.
Do not wipe wells with paper towel.
7) “6, Chromogen” should be stored in the dark due to its sensitivity against light. “6, Chromogen” should be avoided contact with metals.
8) Measurement should be done within 30 minutes after addition of “7, Stop solution”.

CALCULATION OF TEST RESULT

Subtract the absorbance of test sample blank from all data, including standards and unknown samples before plotting. Plot the subtracted absorbance of the standards against the standard concentration on log-log graph paper. Draw the best smooth curve through these points to construct the standard curve. Read the concentration for unknown samples from the standard curve.

Example of standard curve

Conc. (ng/mL)	Absorbance (450nm)
12.5	2.940
6.25	1.639
3.13	0.862
1.56	0.458
0.78	0.241
0.39	0.136
0.20	0.081
0 (Test Sample Blank)	0.025



＊ The typical standard curve is shown above. This curve can not be used to derive test results. Please run a standard curve for each assay.

PERFORMANCE CHARACTERISTICS

1. Titer Assay (Samples with standard added are used.)

Specimen	Titer (x)	Measurement Value (ng/mL)	Theoretical Value (ng/mL)	%
Rat Serum (SD)	400	1.99	2.17	91.7
	800	1.02	1.13	90.3
	1,600	0.52	0.55	94.5
Rat Plasma (EDTA) (SD)	400	2.37	2.57	92.2
	800	1.24	1.30	95.4
	1,600	0.64	0.63	101.6
Mouse Serum (BALB/c)	400	3.31	3.39	97.6
	800	1.64	1.73	94.8
	1,600	0.79	0.86	91.9
Mouse Plasma (EDTA) (BALB/c)	400	3.19	3.27	97.6
	800	1.57	1.65	95.2
	1,600	0.77	0.80	96.3
RPMI-1640	2	6.24	6.25	99.8
	4	2.85	3.13	91.1
	8	1.31	1.56	84.0
Human Serum	400	2.12	2.31	91.8
	800	1.04	1.16	89.7
	1,600	0.55	0.58	94.8
Human Plasma (EDTA)	400	2.03	2.11	96.2
	800	1.04	1.03	101.0
	1,600	0.50	0.52	96.2

2. Added Recovery Assay

Specimen	Theoretical Value (ng/mL)	Measurement Value (ng/mL)	%
Rat Serum (SD) (x400)	2.85	2.78	97.5
	2.07	2.06	99.5
	1.68	1.64	97.6
Rat Plasma (EDTA) (SD) (x400)	2.50	2.35	94.0
	2.11	2.03	96.2
	1.91	1.91	100.0
Mouse Serum (BALB/c) (x800)	2.69	2.40	89.2
	1.90	1.74	91.6
	1.51	1.39	92.1
Mouse Plasma (EDTA) (BALB/c) (x800)	2.57	2.29	89.1
	1.79	1.64	91.6
	1.40	1.31	93.6
RPMI-1640 (x2)	6.25	6.21	99.4
	3.13	2.84	90.7
	1.56	1.49	95.5
Human Serum (x400)	7.81	7.51	96.2
	3.12	2.96	94.9
	1.75	1.72	98.3
Human Plasma (EDTA) (x400)	7.75	7.05	91.0
	3.06	2.84	92.8
	1.69	1.65	97.6

3. Intra - Assay

Measurement Value (ng/mL)	SD value	CV value (%)	n
5.43	0.35	6.4	24
1.21	0.08	6.6	24
0.30	0.03	10.0	24

4. Inter - Assay

Measurement Value (ng/mL)	SD value	CV value (%)	n
6.55	0.23	3.5	3
1.38	0.09	6.8	3
0.31	0.03	8.8	3

5. Sensitivity

44 pg/mL
The sensitivity for this kit was determined using the guidelines under the National Committee for Clinical Laboratory Standards (NCCLS) Evaluation Protocols. (National Committee for Clinical Laboratory Standards Evaluation Protocols, SC1, (1989) Villanova, PA: NCCLS.)

PRECAUTION FOR INTENDED USE AND/OR HANDLING

1. All reagents should be stored at 2-8°C. All reagents shall be brought to room temperature approximately 30 minutes before use.
2. “3, Standard” is lyophilized products. Be careful to open this vial.
3. “7, Stop solution” is a strong acid substance. Therefore, be careful not to have your skin and clothes contact “7, Stop solution” and pay attention to the disposal of “7, Stop solution”.
4. Dispose used materials after rinsing them with large quantity of water.
5. The precipitation may occur in “2, Labeled antibody Conc.”, however, there is no problem in the performance.
6. Wash hands after handling reagents.
7. Do not mix the reagents with the reagents from different lot or different kit.
8. Do not use expired reagents.
9. This kit is for research purpose only. Do not use for clinical diagnosis.

STORAGE AND THE TERM OF VALIDITY

Storage Condition : 2 - 8 °C
The expiry date is specified on outer box.

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Made in Japan.