

Tenascin-C Large (FN III-B) ELISA

Enzyme immunoassay for the quantitative determination of
tenascin-C large (HMV) (FN III-B) in human, mouse and rat serum,
EDTA plasma and cell culture supernatant

REF

JP27767



12 x 8

For illustrative purposes only.

To perform the assay the instructions for use provided with the kit have to be used.

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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 weight variants of Tenascin-C are 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 Affinity Purify
: specific to FNIII-B domain
- Labeled Antibody : HRP conjugated Anti-Tenascin-C (4F10TT) Mouse IgG Fab' Affinity 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 Tenascin-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 subtract its value from the measurement value.

KIT COMPONENT

1	Precoated plate : Anti-Tenascin-C (4C8MS) Mouse IgG MoAb Affinity 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 : 1% BSA, 0.05% Tween 20 in PBS	30mL x 1
5	Solution for Labeled antibody : 1% BSA, 0.05% Tween 20 in PBS	12mL x 1
6	Chromogen : TMB solution	15mL x 1
7	Stop solution : 1N H ₂ SO ₄	12mL x 1
8	Wash buffer Conc. : (40X) 0.05% Tween20 in phosphate buffer	50mL x 1

OPERATION MANUAL

1. Materials needed but not supplied

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

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.)

This operation should be done just before the application of Labeled antibody.

The remaining "2, Labeled antibody Conc." should be stored at 4°C in firmly sealed vial.

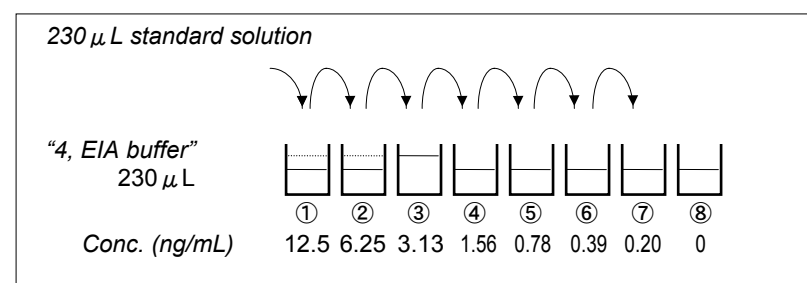
- 3) Preparation of Standard
Put just 0.5 mL of deionized water into the vial of "3, Standard" and mix it gently and completely. This solution is 25 ng/mL Tenascin-C standard.
- 4) Dilution of Standard
Prepare 8 tubes for dilution of "3, Standard". Put 230 μL each of "4, EIA buffer" into the tube.

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				
Washing 7 times				
Labeled Antibody	100 μL	100 μL	100 μL	-
Incubation for 30 minutes at 4°C with plate lid				
Washing 9 times				
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 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 each well of the precoated plate vigorously with wash buffer using washing bottle. Then, fill each well with wash buffer and leave the precoated plate lay for 15~30 seconds. Remove wash buffer completely from the precoated plate by snapping. This procedure must be repeated more than 7 times. Then, remove the remaining liquid from all wells completely by snapping the precoated plate onto paper towel.
In case of using plate washer, after 4 times washing with plate washer, washing with above washing bottle must be repeated 3 times.
- 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 precoated plate 9 times in the same manner above 4).
- 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

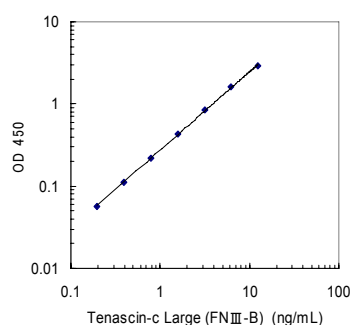
- 1) Test samples should be measured soon after the collection. For storage of test samples, store them frozen and do not repeat freeze/thaw cycles. Thaw the test samples at low temperature and mix them completely before measurement.
- 2) Test samples should be diluted with "4, EIA buffer" or PBS, if 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

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
6.59	0.43	6.5	14
1.39	0.10	7.2	14
0.32	0.03	9.4	14

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. "1, Precoated plate" and "3, Standard" contain sodium azide. Therefore, dispose these materials after diluting them with large quantity of water to avoid the production of explosive metallic azide.
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 term of validity : 12 months

(The expiry date is specified in outer box.)

REFERENCE

1. Jones FS, Jones PL. The tenascin family of ECM glycoproteins: structure, function, and regulation during embryonic development and tissue remodeling. *Dev Dyn.* 2000 Jun; 218(2):235-59.
2. Imanaka-Yoshida K, Hiroe M, Yasutomi Y, Toyozaki T, Tsuchiya T, Noda N, Maki T, Nishikawa T, Sakakura T, Yoshida T. Tenascin-C is a useful marker for disease activity in myocarditis. *J Pathol.* 2002 Jul; 197(3):388-94.
3. Sato M, Toyozaki T, Odaka K, Uehara T, Arano Y, Hasegawa H, Yoshida K, Imanaka-Yoshida K, Yoshida T, Hiroe M, Tadokoro H, Irie T, Tanada S, Komuro I. Detection of experimental autoimmune myocarditis in rats by 111In monoclonal antibody specific for tenascin-C. *Circulation.* 2002 Sep 10;106(11):1397-402.
4. Imanaka-Yoshida K, Hiroe M, Nishikawa T, Ishiyama S, Shimojo T, Ohta Y, Sakakura T, Yoshida T. Tenascin-C modulates adhesion of cardiomyocytes to extracellular matrix during tissue remodeling after myocardial infarction. *Lab Invest.* 2001 Jul; 81(7):1015-24.
5. Yoshida T, Matsumoto E, Hanamura N, Kalembeiy I, Katsuta K, Ishihara A, Sakakura T. Co-expression of tenascin and fibronectin in epithelial and stromal cells of benign lesions and ductal carcinomas in the human breast. *J Pathol.* 1997 Aug; 182 (4):421-8.
6. Tsunoda T, Inada H, Kalembeiy I, Imanaka-Yoshida K, Sakakibara M, Okada R, Katsuta K, Sakakura T, Majima Y, Yoshida T. Involvement of large tenascin-C splice variants in breast cancer progression. *Am J Pathol.* 2003 Jun;162 (6):1857-67.
7. Sato A, Aonuma K, Imanaka-Yoshida K, Yoshida T, Isobe M, Kawase D, Kinoshita N, Yazaki Y, Hiroe M. Serum tenascin-C might be a novel predictor of left ventricular remodeling and prognosis after acute myocardial infarction. *J Am Coll Cardiol.* 2006 Jun 6; 47 (11):2319-25.
8. Tanaka H, El-Karef A, Kaito M, Kinoshita N, Fujita N, Horie S, Watanabe S, Yoshida T, Adachi Y. Circulating level of large splice variants of tenascin-C is a marker of piecemeal necrosis activity in patients with chronic hepatitis C. *Liver Int.* 2006 Apr; 26 (3):311-8.
9. Hasegawa M, Sudo A, Nagakura T, Hirata H, Kinoshita N, Yoshida T, Uchida A. Tenascin-C levels in pseudosynovial fluid of loose hip prostheses. *Scand J Rheumatol.* 2005 Nov-Dec; 34 (6):464-8.
10. Morimoto S, Imanaka-Yoshida K, Hiramitsu S, Kato S, Ohtsuki M, Uemura A, Kato Y, Nishikawa T, Toyozaki T, Hishida H, Yoshida T, Hiroe M. Diagnostic utility of tenascin-C for evaluation of the activity of human acute myocarditis. *J Pathol.* 2005 Mar; 205 (4):460-7.
11. Hasegawa M, Hirata H, Sudo A, Kato K, Kawase D, Kinoshita N, Yoshida T, Uchida A. Tenascin-C concentration in synovial fluid correlates with radiographic progression of knee osteoarthritis. *J Rheumatol.* 2004 Oct; 31 (10):2021-6.
12. Imanaka-Yoshida K, Hiroe M, Yoshida T. Interaction between cell and extracellular matrix in heart disease: multiple roles of tenascin-C in tissue remodeling. *Histol Histopathol.* 2004 Apr; 19 (2):517-25.
13. Hanamura N, Yoshida T, Matsumoto E, Kawarada Y, Sakakura T. Expression of fibronectin and tenascin-C mRNA by myofibroblasts, vascular cells and epithelial cells in human colon adenomas and carcinomas. *Int J Cancer.* 1997 Sep 26; 73(1):10-5.

Version

070222 Established

Symbols / Symbole / Symbôles / Símbolos / Símbolos / Σύμβολα

	Cat.-No.: / Kat.-Nr.: / No.- Cat.: / Cat.-No.: / N.º Cat.: / N.-Cat.: / Αριθμός-Κατ.:
	Lot-No.: / Chargen-Bez.: / No. Lot: / Lot-No.: / Lote N.º: / Lotto n.: / Αριθμός -Παραγωγή:
	Use by: / Verwendbar bis: / Utiliser à: / Usado por: / Usar até: / Da utilizzare entro: / Χρησιμοποιείται από:
	No. of Tests: / Kitgröße: / Nb. de Tests: / No. de Determ.: / N.º de Testes: / Quantità dei tests: / Αριθμός εξετάσεων:
	Concentrate / Konzentrat / Concentré / Concentrar / Concentrado / Concentrato / Συμπύκνωμα
	Lyophilized / Lyophilisat / Lyophilisé / Liofilizado / Liofilizado / Liofilizzato / Λυοφιλισμένο
	In Vitro Diagnostic Medical Device. / In-vitro-Diagnostikum. / Appareil Médical pour Diagnostics In Vitro. / Dispositivo Médico para Diagnóstico In Vitro. / Equipamento Médico de Diagnóstico In Vitro. / Dispositivo Medico Diagnostico In vitro. / Ιατρική συσκευή για In-Vitro Διάγνωση.
	Evaluation kit. / Nur für Leistungsbewertungszwecke. / Kit pour évaluation. / Juego de Reactivos para Evaluació. / Kit de avaliação. / Kit di valutazione. / Κιτ Αξιολόγησης.
	Read instructions before use. / Arbeitsanleitung lesen. / Lire la fiche technique avant emploi. / Lea las instrucciones antes de usar. / Ler as instruções antes de usar. / Leggere le istruzioni prima dell'uso. / Διαβάστε τις οδηγίες πριν την χρήση.
	Keep away from heat or direct sun light. / Vor Hitze und direkter Sonneneinstrahlung schützen. / Garder à l'abri de la chaleur et de toute exposition lumineuse. / Manténgase alejado del calor o la luz solar directa. / Manter longe do calor ou luz solar directa. / Non esporre ai raggi solari. / Να φυλάσσεται μακριά από θερμότητα και άμεση επαφή με το φως του ηλίου.
	Store at: / Lagern bei: / Stocker à: / Almacene a: / Armazemar a: / Conservare a: / Αποθήκευση στους:
	Manufacturer: / Hersteller: / Fabricant: / Productor: / Fabricante: / Fabricante: / Παραγωγός:
	Caution! / Vorsicht! / Attention! / ¡Precaución! / Cuidado! / Attenzione! / Προσοχή!
<p>Symbols of the kit components see MATERIALS SUPPLIED. Die Symbole der Komponenten sind im Kapitel KOMPONENTEN DES KITS beschrieben. Voir MATERIEL FOURNI pour les symbôles des composants du kit. Símbolos de los componentes del juego de reactivos, vea MATERIALES SUMINISTRADOS. Para símbolos dos componentes do kit ver MATERIAIS FORNECIDOS. Per i simboli dei componenti del kit si veda COMPONENTI DEL KIT. Για τα σύμβολα των συστατικών του κιτ συμβουλευτείτε το ΠΑΡΕΧΟΜΕΝΑ ΥΛΙΚΑ.</p>	

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LIABILITY: Complaints will be accepted in each mode –written or vocal. Preferred is that the complaint is accompanied with the test performance and results. Any modification of the test procedure or exchange or mixing of components of different lots could negatively affect the results. These cases invalidate any claim for replacement. Regardless, in the event of any claim, the manufacturer's liability is not to exceed the value of the test kit. Any damage caused to the kit during transportation is not subject to the liability of the manufacturer.