Code No. 27121

Human Fibulin-5/DANCE Assay Kit - IBL

INTRODUCTION

DANCE was identified as a ligand of integrins expressed mainly in arteries during embryogenesis and was named developmental arteries and neural crest EGF-like /DANCE (ref. 1). It is also called fibulin-5 and is a member of fibulin-family (ref. 3).
Fibulin-5/DANCE is a secreted protein of 448 amino acids and 66kDa. It contains a RGD (arginine-glycine-asparticacid) motif in the N-terminal ragion and binds to integrins α5β1, αvβ3 and αvβ5. The expression of mRNA is augmented in smooth muscle cell, fibroblasts and endothelial cells of injured vascular tissues.
Fibulin-5-deficient mice recapitulate human aging phenotypes, such as loose skin (cutis laxa), emphysematous lungs, and stiff ateries. It is known that destruction and degradation of elastic fibers directly cause many aging-related disorders like these. Disorganized elastic fibers were observed in the fibulin-5-deficient mice and indicating that fibulin-5/DANCE is an essential protein for elastogenesis (ref. 2).
This product is an ELISA kit wich can measure human fibulin-5/DANCE.

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 quantities of Human Fibulin-5/DANCE.

MEASUREMENT RANGE

0.08 - 5.0 ng/mL

INTENDED USE

For research use only, not for use in diagnostic procedures.

- This IBL's assay kit is capable for the quantitative determination of human fibulin-5/DANCE in EDTA-plasma and cell culture supernatant.
- The guide line of dilution rate for plasma samples is about 200-fold with "4, EIA buffer.

KIT COMPONENT

1	Precoated plate	: Anti-DANCE Mouse IgG MoAb Affinity Purify	96Well x 1
2	Labeled antibody Conc.	:	
	(30X) HRP conjugated Anti-DANCE Mouse IgG MoAb Fab' Affinity Purify		0.4mL x 1
3	Standard	: Recombinant human DANCE	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)
- 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 50 mL of "8, Wash buffer Conc." with 1,950 mL 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 strip (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 applying labeled antibody.</u>
The remaining "2, Labeled antibody Conc." should be stored at 4°C in firmly sealed vial.

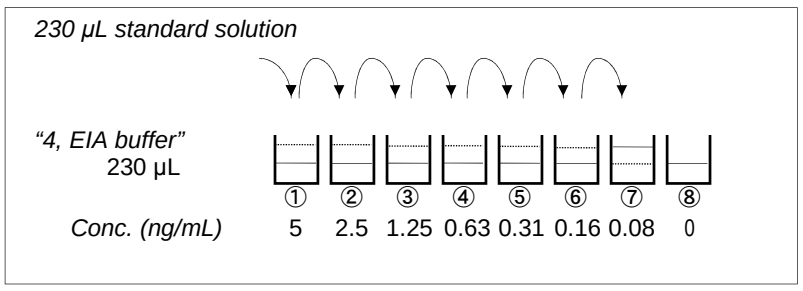
3) Preparation of Standard
Put just <u>0.5 mL</u> of deionized water into the vial of "3, Standard" and mix it gently and completely. Concentration of the standard is 10 ng/mL.

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	5 ng/mL
Tube-2	2.5 ng/mL
Tube-3	1.25 ng/mL
Tube-4	0.63 ng/mL
Tube-5	0.31 ng/mL
Tube-6	0.16 ng/mL
Tube-7	0.08 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 5 ng/mL and 0.08 ng/mL. Tube-8 is the test sample blank as 0 ng/mL.

See following picture.



5) Dilution of test sample
<u>Plasma samples have to be diluted with "4, EIA buffer" accordingly.
The recommended dilution for them is about 200-fold. In case of the absorbance of sample is over than the assay range, it is necessary to dilute it more.</u>

<u><Example of 200-fold dilution of plasma></u>
1. Add 10 µL of plasma to 190 µL of "4, EIA buffer" in a tube and mix them well.
2. Pipette 30 µL of 20-fold diluted plasma from the tube of above first dilution and add it to 270 µL of "4, EIA buffer" in another tube, and mix them well.
3. This 200-fold diluted plasma should be applied as a test sample according to the measurement procedure.

When "4, EIA buffer" in kit is not enough for dilution, customers can purchase additional kit component (30 mL, Code No. 27121D).

3. Measurement procedure

All reagents shall be brought to room temperature approximately 30 minutes before use. Then mix it gently and completely before use. Make sure of 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) Take the required quantity of "6, Chromogen" and put it into a disposable test tube. Then, pipette 100 µL from the test tube into every well. Please do not return the rest of used chromogen in the test tube into "6, Chromogen" bottle in order to avoid contamination.
9) Incubate the precoated plate for 30 minutes at room temperature in the dark. The solution of Chromogen will turn blue.
10) Add 100 µL of "7, Stop solution" to all wells. Mix the solution by tapping the side of precoated plate. The solution will turn yellow by 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 solution. Then, run the plate reader and conduct measurement at 450 nm against a reagent blank.
The measurement shall be done within 30 minutes after addition of "7, Stop solution".

SPECIAL ATTENTION

1) Test samples should be measured soon after collection. For the storage of test samples, store them frozen and do not repeat freeze/thaw cycles. Thaw the test samples at a low temperature and mix them completely before measurement.
2) Test samples should be diluted with "4, EIA buffer", suitably.
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 in this kit for washing the precoated plate. Insufficient washing may lead to the failure in measurement.

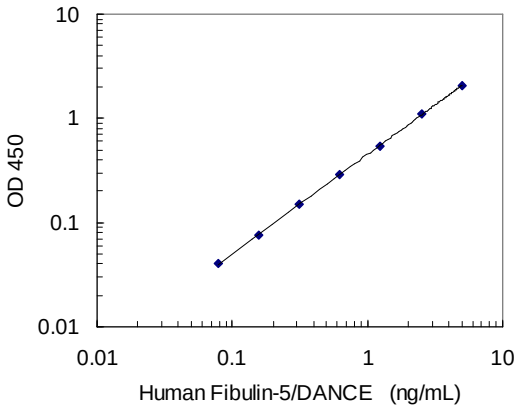
6) Remove the wash buffer completely by tapping the precoated plate on paper towel. Do not wipe wells with paper towel.
7) "6, Chromogen" should be stored in the dark due to its sensitivity against light. Avoid contact of Chromogen with metals.
8) Measurement should be done within 30 minutes after addition 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

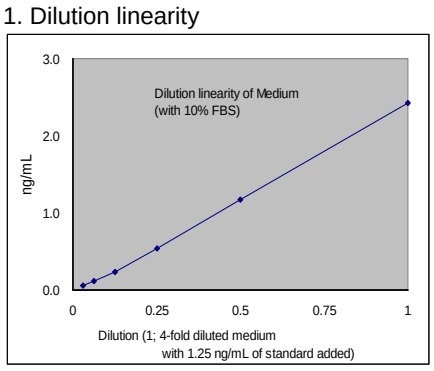
Conc. (ng/mL)	Absorbance (450nm)
5	2.096
2.5	1.102
1.3	0.542
0.63	0.295
0.31	0.156
0.16	0.081
0.08	0.045
0 (Test Sample Blank)	0.005

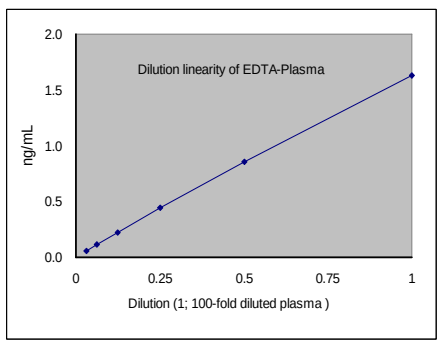


* The typical standard curve is shown above. This curve can not be used to derive test results. Please run a standard curve for each assay.

PERFORMANCE CHARACTERISTICS

1. Dilution linearity





2. Added Recovery Assay

Specimen	Additive Amount (ng/mL)	Theoretical Value (ng/mL)	Measured Value (ng/mL)	%
Human Plasma (EDTA) (x200)	2.50	3.29	3.49	106.1
	1.25	2.04	1.77	86.5
	0.63	1.42	1.35	95.1
	0.31	1.10	1.01	91.6
Medium with 10% FBS (x4)	2.50	3.61	3.67	101.7
	1.25	2.36	2.20	93.1
	0.63	1.73	1.43	82.7
	0.31	1.42	1.15	80.7

3. Intra – Assay

Mean Value (ng/mL)	SD (ng/mL)	CV (%)	n
0.834	0.049	5.9	24
0.298	0.021	7.0	24
0.091	0.008	8.8	24

4. Inter – Assay

Mean Value (ng/mL)	SD (ng/mL)	CV (%)	n
1.101	0.080	7.3	5
0.507	0.066	13.0	5
0.248	0.026	10.5	5

5. Sensitivity

0.01 ng/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. Precipitation may occur in "2, Labeled antibody Conc.", "4, EIA buffer" or "8, Wash buffer 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 a different lot or 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.

REFERENCE

1. Nakamura T, Ruiz-Lozano P, Lindner V, Yabe D, Taniwaki M, Furukawa Y, Kobuke K, Tashiro K, Lu Z, Andon NL, Schaub R, Matsumori A, Sasayama S, Chien KR, Honjo T. DANCE, a novel secreted RGD protein expressed in developing, atherosclerotic, and balloon-injured arteries. J Biol Chem. 1999 Aug 6;274(32):22476-83.
2. Nakamura T, Lozano PR, Ikeda Y, Iwanaga Y, Hinek A, Minamisawa S, Cheng CF, Kobuke K, Dalton N, Takada Y, Tashiro K, Ross Jr J, Honjo T, Chien KR. Fibulin-5/DANCE is essential for elastogenesis in vivo. Nature. 2002 Jan 10;415(6868):171-5.
3. Yanagisawa H, Davis EC, Starcher BC, Ouchi T, Yanagisawa M, Richardson JA, Olson EN. Fibulin-5 is an elastin-binding protein essential for elastic fibre development in vivo. Nature. 2002 Jan 10;415(6868):168-71.
4. Hirai M, Ohbayashi T, Horiguchi M, Okawa K, Hagiwara A, Chien KR, Kita T, Nakamura T. Fibulin-5/DANCE has an elastogenic organizer activity that is abrogated by proteolytic cleavage in vivo. J Cell Biol. 2007 Mar 26;176(7):1061-71.

*This product has been jointly-developed with NB Health Laboratory Co. Ltd. and Kansai Medical University.

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Made in Japan.