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Catalog #KDT-010/E

Dityrosine (DT) ELISA kit

Dityrosine (DT) is known to be formed when tyrosine is damaged by free radicals, and is reported to exist in various tissues and is also detected in urine. DT ELISA kit is designed for quantitative measurement of DT especially in urine samples.
For research use only. Not for diagnostic nor medical use.

1. Kit contents.

Item	Description	Quantity
1) DT Microtiter Plate	: Pre-coated with DT (8x 12 wells, split type)	1 plate
2) Primary Antibody	: Anti DT monoclonal antibody (ready to use)	1 vial (7 mL)
3) Secondary Antibody	: HRP-conjugated anti-mouse antibody	1 vial
4) Secondary Antibody Solution	: Phosphate Buffered Saline	1 vial (12 mL)
5) TMB Substrate	: Chromogen (ready to use)	1 vial (12 mL)
6) Stop Solution	: 1.96% Sulfuric acid (ready to use)	1 vial (12 mL)
7) Washing Solution (x5)	: Concentrated wash buffer	3 vials (25 mL x 3)
8) DT Standards	: DT standards (ready to use) (**A:** 0.05, **B:** 0.2, **C:** 0.5, **D:** 2, **E:** 5, **F:** 12 μmol/L)	1 vial each (0.5 mL)
9) Plate seal	:	2 sheets

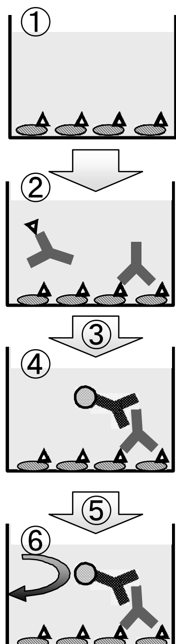
*Storage conditions: Store at 2-8℃. **Don't freeze**.
*Expiration : 1 year. After the vials are opened, the kit should be used in one week.
*Measuring range : 0.05 - 12 μmol/L

2. Required but not provided.

1) Distilled water (Preparation of washing solution)
2) Saline (0.9％ NaCl in distilled water）for sample dilution
3) 50 micro L micropipettor and pipette tips
4) 8-channel (50-200 micro L) micropipettor and tips.
5) Reagent trays for 8-channel micropipettor.
6) 4-10 degree C incubator.
7) Microtiter plate reader (measuring wavelength 450 nm).

3. Principles of the procedure.

[Fig.1]



1) Prepare microtiter plate pre-coated with dityrosine (DT).

2) Add DT standard solution or sample to microtiter plate well, and subsequently add anti DT monoclonal antibody. The DT in the standard or sample competes with the DT on the well surface for the anti DT antibody. As a result, higher concentration of DT in sample will result in reduced binding of the antibody bound to the surface of the well.

3) The antibody bound to the DT in sample, is removed from the well by washing. While the antibody bound to pre-coated DT remain on the surface of the well.

4) Peroxidase-conjugated secondary antibody is added to the well, and binds to the anti DT antibody.

5) Unbound secondary antibody is removed by washing.

6) Addition of the chromatic reagent results in the development of color in proportion to the amount of antibody bound to the well. The reaction is terminated by stop solution. Absorbance at 450 nm is measured using microtiter plate reader.

7) Draw a calibration curve from the absorbance data of standards, and calculate the concentration of DT in the sample.

4. Sample pretreatment.

Application to human urine:
Mix with 3 volumes of saline before assay. Insoluble materials should be removed by centrifugation.
Please note that samples must be diluted with saline (0.9% NaCl in distilled water).
Washing buffer and PBS can't be used for sample dilution.

5. Assay Procedure.

Bring all reagents, samples and microtiter plate to room temperature before use. Pick out ①*Microtiter plate* from the bag. To use some wells at the next experiment, remove the well splits from the frame, put it into the bag, and store at 4 degree C. ①*Microtiter plate* is stable for 1 week after opening the bag.

A) Mix 1 vial of ⑦*Washing solution* with 100 mL of distilled water, and prepare *working washing buffer*.

B) Put ①*Microtiter plate* from the bag. Inside wells of ①*Microtiter plate*, glycerol is coated as a preservative. Remove seal from wells before use.

C) Pour 250 µL of working washing buffer, stand for 3 minutes at room temperature, and remove buffer from wells. Remove the remaining solution by tapping the plate against clean paper towel. Repeat washing for additional two times.

D) Pour 50 µL of ⑧*DT standards* or diluted samples. For blank wells, pour 100 µL of distilled water. The typical layout of microtiter plate is shown in Fig.2

E) Pour 50 µL of ②Primary antibody to all wells expect blank wells. Seal the microtiter plate tightly with ⑨*Plate Seal*. Mix gently by shaking the microtiter plate horizontally. Incubate at 4 - 10 degree C for overnight. Store all reagents at 4 degree C.

F) Bring all reagents expect working microtiter plate to room temperature.

G) Prepare working washing buffer. Mix 1 vial of ⑦*Washing solution* with 100 mL of distilled water. Working washing buffer is stable for one week at 4 degree C.

H) Reconstitute working secondary antibody. Add 1 vial of ④*Secondary Antibody Buffer to* ③*Secondary Antibody*, mix gently and stand for 5 minutes at room temperature. Working secondary antibody is stable for 1 week at 4 degree C.

I) Remove the plate seal, and pour off the contents of mictotiter plate by turn the plate upside down. The use of aspirator is not recommended. Remove the remaining solution by tapping the plate against clean paper towel. Add 250 µL of working washing buffer to all well, mix gently by horizontal shaking, and remove the contents similarly. Repeat washing procedure twice and remove the remaining solution of the well.

J) Pour 100 µL of working secondary antibody to all well. Seal the microtiter plate tightly with ⑨*Plate Seal*. Mix gently by shaking the microtiter plate horizontally. Incubate at room temperature (20-24 degree C) for 1 hou

K) Remove the plate seal, and wash the plate as mentioned at STEP I for 3 times. Remove the remaining solution of the well.

L) Pour 100 µL of ⑤*TMB substrate* to all well, and incubate at room temperature for 15 minutes in the dark. ⑤ *TMB substrate* is light sensitive. Avoid room light by covering the working microtiter plate with aluminium foil.

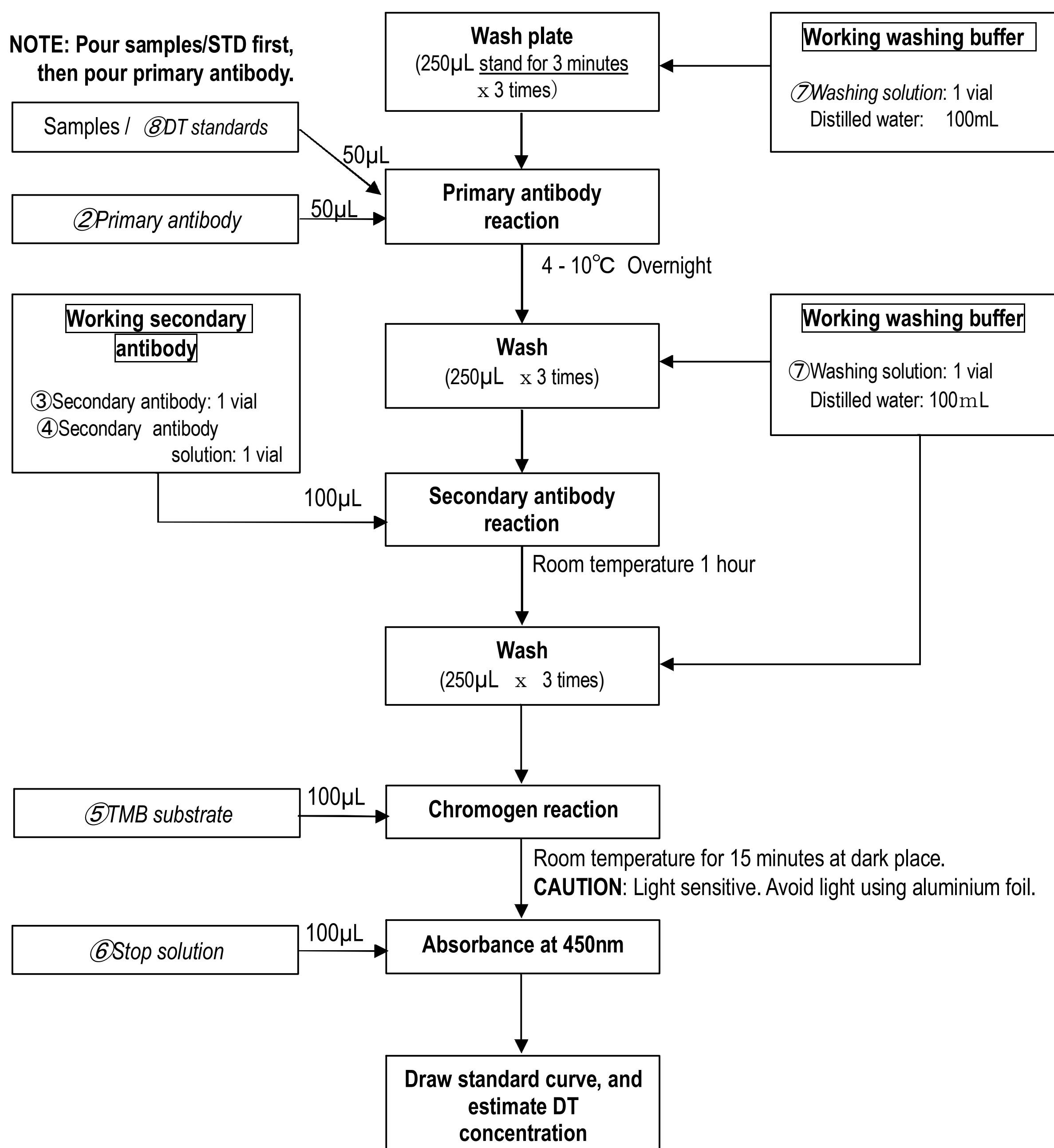
M) Add 100 µL of ⑥*Stop Solution* to all well, mix gently, wait for 3 minutes, and measure the absorbance at 450 nm.

[Fig.2 Typical layout of microtiter plate for triplicate assay.]

To avoid edge effects, the use of outmost wells (Rows A and H, marked as "X") is not recommended. 54 wells (18 samples x 3) are applicable for test samples.

	1	2	3	4	5	6	7	8	9	10	11	12
A	Blank wells(n=3)			X	X	X	X	X	X	X	X	X
B	⑧*Standard-A*			Sample-1			Sample-7			Sample-7		
C	⑧*Standard-B*			Sample-2			Sample-8			Sample-8		
D	⑧*Standard-C*			Sample-3			Sample-9			Sample-9		
E	⑧*Standard-D*			Sample-4			Sample-10			Sample-10		
F	⑧*Standard-E*			Sample-5			Sample-11			Sample-11		
G	⑧*Standard-F*			Sample-6			Sample-12			Sample-12		
H	X	X	X	X	X	X	X	X	X	X	X	X

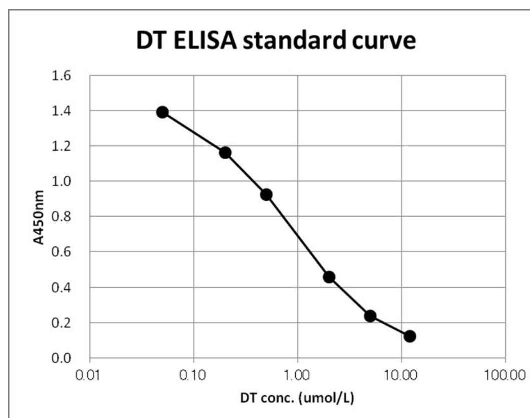
6. Procedure flow chart.



7. Standard curve and calculations.

Generate the standard curve by plotting absorbance vertical axis and log of concentration as the horizontal axis. An example is shown in Fig.3. Any smooth curve fit may be applicable. Please note that the standard curve should be established for every assay.

[Fig.3] A standard curve example.



8. Reference.

Kato Y, Wu X, Naito M, Nomura H, Kitamoto N, Osawa T.: Immunochemical detection of protein dityrosine in atherosclerotic lesion of apo-E-deficient mice using a novel monoclonal antibody. Biochem Biophys Res Commun. 275(1), p11-15 (2000).

9. Technical support & troubleshooting

A) Unstableness of incubation temperature may result in unstable results.
B) Residual water drop in wells may cause unstable results. Please remove water drop completely by tapping microtiter plate to clean and dry paper towel. Please take care not to touch inside wells.
C) Accuracy in pipetting volume for samples and reagents may affect the quality of assay. Please note that samples, standards and primary antibody must be poured accurately 50 µL.
D) Temperature for secondary antibody reaction and chromogen reaction may affect absorbance. Please try to extend or shorten chromogen reaction time depending on room temperature.
E) TMB substrate is light sensitive. Room light may cause spontaneous / non-specific coloring. Avoid sun light and room light by covering TMB substrate vial and microtiter plate with aluminium foil.
F) Proteins, high concentration of salts and high / low pH in samples may affect ELISA. Please remove proteins by ultrafiltration, dilute samples using saline, adjust pH to 8.0±0.5.
G) For sample dilution, washing solution, working washing buffer or phosphate buffered saline (pH7.4) can't be used. Please use saline (0.9% NaCl in distilled water)

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or **FAX: +81-538-49-1267**
(Supported languages: Japanese and English)

Japan Institute for the Control of Aging (JaICA),
Nikken SEIL co., Ltd.
Haruoka 710-1, Fukuroi, Shizuoka 437-0122, Japan
TEL: +81-538-49-0125 FAX: +81-538-49-1267
www.jaica.com/e/ E-mail: biotech@jaica.com