Instructions for Use

DxMe® BC Kit (16-Well)

Key to Symbols Used

IVD	In vitro diagnostic medical device	REF	Catalogue number
	Do not re-use	LOT	Batch code
Σ 96	Content sufficient for 96 tests		Consult instructions for use
	Manufacturer	2-8°C	Storage temperature
CE	CE labeled		Expiration date
EC REP	Manufacturer authorized representative in the European community		

Warning: DxMe® BC kit immunoassay results obtained with different lots or assays cannot be used reciprocally due to differences in reagent reactivity. The results, which must be obtained and analyzed by certified medical professionals, must contain a statement identifying which DxMe® BC kit was used. DxMe® BC test results can aid in the diagnosis of breast carcinoma but cannot be used as the only method of examination.

Contents

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Intended Use

DxMe® BC kit aids the diagnosis of breast malignant tumors by analyzing the concentration of Trx1 in the samples (sera) of women using enzyme-linked immunosorbent assay(ELISA). As DxMe® BC kit is intended to aid in the diagnosis of breast cancer, which is performed by comprehensive analysis of the results of radiological and histological tests by providing information that is helpful in diagnosing breast cancer.

※ This test shall not be used to determine whether breast cancer screening or mammography is needed.

Summary

The test values from the DxMe® BC kit are defined by the use of the monoclonal antibodies (MAb) AB1 and AB2 in a sandwiched ELISA. AB1 and AB2 specific determinants detected by the test are defined as an antioxidative protein having a molecular weight of 12.6 kDa. This protein is thioredoxin 1 (Trx1), which is a member of the thioredoxin superfamily [1 - 9] Trx1 is normally found in the oxidative electron transport system and exists at a very low concentration in the blood. It is known that, when cells become malignant, Trx1 is secreted into serum, increasing its concentration. As a major factor in in vivo antioxidant systems, Trx1 has consistently stood out as an object of research on oncological mechanisms and cancer treatment. It is shown that the gene expression level of Trx1 is lower in normal breast tissue than in breast cancer tissue. The level of Trx1 in the blood of healthy women is known to be very low, whereas it increases significantly in breast cancer patients due to the secretion of Trx1 out of the cancer cells. These specific conditions make it possible to detect breast cancer by measuring the level of Trx1 in serum. The DxMe® BC Kit is not recommended as a sole tool to confirm breast cancer. However, it can be used as an additional diagnostic aid for breast cancer.

Basic Principles

DxMe® BC utilizes the ‘sandwich’ enzyme-linked immunosorbent assay (ELISA) technique. It uses two highly specific monoclonal antibodies to quantitatively measure the Trx1 level in blood serum. First, the sample (serum) or the purified Trx1 protein (Calibrator, Positive Control and Negative Control) is added to wells of a 96-well plate coated with anti-Trx1 monoclonal antibody, and the antibody and the antigen are left to bind to each other for a given time. After washing, the detection antibody (anti-Trx1 monoclonal antibody conjugated with peroxidase) is applied to the wells to form a coated antibody-Trx1-peroxidase conjugated antibody complex. The reaction mixture is then colored by the reaction between the antibody-coupled peroxidase and its substrate TMB. The coloring reaction is stopped by sulfuric acid, which turns the blue color into yellow. After the reaction completely stops, the intensity of the color is measured by a microplate reader at 450 nm. The relative level of Trx1 in the sample serum can be quantitatively calculated by the degree of light absorption.

Composition of Reagents

No.	Name	Purpose	Component Name	Amount	Standard	Remarks
1	Plate-B (Antibody Coated Plate)	Coated with 1st antibody	Anti-Trx1 Monoclonal IgG	1.6 μg	Self Std	16 well /Plate
		plate	Nunc Immuno F8 Module, MaxiSorp	1 ea	85.40 x 127.60 x 14.40 mm	
2	Calibrator, C1(40 ng/㎖)	Standard material	Thioredoxin1, Human	12ng	Self Std	0.3 ㎖/vial
		Solvent	Phosphate Buffered Saline pH7.4, contains BSA	5.865㎎	Self Std	
		Solvent	H_2O	0.3㎖	KP	
3	Calibrator, C2 (20 ng/㎖)	Standard material	Thioredoxin1, Human	6ng	Self Std	0.3 ㎖/vial
		Solvent	Phosphate Buffered Saline pH7.4, contains BSA	5.865㎎	Self Std	
		Solvent	H_2O	0.3㎖	KP	
4	Calibrator, C3 (10 ng/㎖)	Standard material	Thioredoxin1, Human	3ng	Self Std	0.3 ㎖/vial
		Solvent	Phosphate Buffered Saline pH7.4, contains BSA	5.865㎎	Self Std	
		Solvent	H_2O	0.3㎖	KP	
5	Calibrator, C4 (5 ng/㎖)	Standard material	Thioredoxin1, Human	1.5ng	Self Std	0.3 ㎖/vial
		Solvent	Phosphate Buffered Saline pH7.4, contains BSA	5.865㎎	Self Std	
		Solvent	H_2O	0.3㎖	KP	
6	Calibrator, C5 (2.5 ng/㎖)	Standard material	Thioredoxin1, Human	0.75ng	Self Std	0.3 ㎖/vial
		Solvent	Phosphate Buffered Saline pH7.4, contains BSA	5.865㎎	Self Std	
		Solvent	H_2O	0.3㎖	KP	
7	Calibrator, C6 (0 ng/㎖)	Standard material	Thioredoxin1, Human	0ng	Self Std	0.3 ㎖/vial
		Solvent	Phosphate Buffered Saline pH7.4, contains BSA	5.865㎎	Self Std	
		Solvent	H_2O	0.3㎖	KP	
8	Detection Antibody (1000x)	2nd antibody	Anti-Trx1 Monoclonal Antibody 2	0.15 μg	Self Std	0.015 ㎖ /vial
		Solvent	Phosphate Buffered Saline pH7.4, contains BSA	0.29325㎎	Self Std	
		Solvent	H_2O	0.015㎖	KP	
9	Positive Control	Standard material	Thioredoxin1, Human	6ng	Self Std	0.3 ㎖/vial
		Solvent	Phosphate Buffered Saline pH7.4, contains BSA	5.865㎎	Self Std	
		Solvent	H_2O	0.3㎖	KP	
10	Negative Control	Standard material	Thioredoxin1, Human	1.5ng	Self Std	0.3 ㎖/vial
		Solvent	Phosphate Buffered Saline pH7.4, contains BSA	5.865 mg	Self Std	
		Solvent	H_2O	0.3㎖	KP	
11	Reagent Dilution Buffer	Dilution material	Phosphate Buffered Saline pH7.4, contains BSA	70.38㎎	Self Std	3.6 ㎖/vial
		Solvent	H_2O	3.6㎖	KP	
12	Washing Solution (10x)	Washing solution	Phosphate Buffered Saline pH7.4, contains TWEEN20	30㎎	Self Std	3 ㎖/vial
		Solvent	H_2O	3㎖	KP	
13	TMB Solution	Color reaction	3,3‘,5,5’-Tetramethylbenzidine	3.6㎖	Self Std	3.6 ㎖/vial
14	STOP Solution	Stopping immune reaction	Sulfuric acid	0.2㎖	USP	3.6 ㎖/vial
		Solvent	H_2O	3.4㎖	KP	

Std: Standard, KP: Korea Pharmacopoeia, USP United States Pharmacopoeia

Contents of the DxMe® BC Kit

Components	16 test	
Plate-B (Antibody Coated 96-well Plate)	1EA	16 WELL
Calibrator, C1 (40 ng/㎖)	1 Vial	0.3 ㎖
Calibrator, C2 (20 ng/㎖)	1 Vial	0.3 ㎖
Calibrator, C3 (10 ng/㎖)	1 Vial	0.3 ㎖
Calibrator, C4 (5 ng/㎖)	1 Vial	0.3 ㎖
Calibrator, C5 (2.5 ng/㎖)	1 Vial	0.3 ㎖
Calibrator, C6 (0 ng/㎖)	1 Vial	0.3 ㎖
Detection Antibody (1000x) (DA)	1 Vial	0.015 ㎖
Positive Control (PC)	1 Vial	0.3 ㎖
Negative Control (NC)	1 Vial	0.3 ㎖
Reagent Dilution Buffer (RD)	1 Bottle	15 ㎖
Washing Solution (10x) (WS)	1 Bottle	20 ㎖
TMB Solution (TM)	1 Bottle	11 ㎖
STOP Solution (SS)	1 Bottle	11 ㎖
Plastic Cover	1EA	-

Materials Required but Not Provided

1) A calibrated microplate reader (450 nm)
2) A calibrated incubator (25°C) and 96 well shaker
3) Calibrated variable precision micropipettes
4) Paper towels as absorbents
5) Distilled water (DW)
6) A timer
7) Graph paper or proper software for data reduction
8) Reservoirs for multi-channel micropipettes
9) Aluminum foil

Storage and Expiry Date

Unopened DxMe® BC Kit are stable until the expiration date on the label when stored at 2-8℃.
DxMe® BC Kit stability has been demonstrated for 6 months from manufacturing.

Precautions and Warnings

1. General Precautions

1) DxMe® BC Kit is an *in vitro* diagnostic device.
2) DxMe® BC Kit is intended for use by laboratory professionals.
3) DxMe® BC Kit is for single use only.
4) Before testing, make sure to read the instructions for use (IFU) and follow the provided protocols.
5) Best results are obtained from regularly calibrated equipment.

2. Safety Precautions

1) It is recommended to put on safety goggles, gloves, and a lab coat when using the kit.
2) Please contact the manufacturer to inquire about hazardous materials.
3) Be careful to avoid contact with the skin or eyes when using the Stop Solution containing 2 N sulfuric acid.

3. Serum Preparation Precautions

1) Only human serum (collected in serum separator tubes [SST]) can be used with the DxMe® BC Kit.
2) When collecting blood by venipuncture, do not use EDTA coated plasma separating tubes or similar ones.
3) After the centrifugation (3,000 rpm, 15 min), separated serum must be aliquoted into fresh 1.5 ㎖ tubes immediately. They should be stored frozen at -70°C or colder.
4) The DxMe® BC Kit does not provide the capability to verify sample type. It is the responsibility of the operator to verify that the correct sample is used in the test.
5) Use caution when handling patients' samples to avoid cross contamination. Use of disposable pipettes or sterilized pipette tips is strongly recommended.
6) Do not use samples suspected of being hemolyzed.
7) Do not use any additionally processed samples, such as heat-inactivated.
8) Serum should be free of lipid, fibrin, red blood cells, or other particles.
9) If serum requires long-term storage, Serum should be stored frozen at -70°C or colder.
10) The freezing and thawing cycle of serum shall not exceed 10 cycle. Serum samples must be homogenized thoroughly after thawing, by low speed vortexing or gentle inverting, and centrifuged prior to use to remove red blood cells or particles and ensure the consistency of samples.
11) For shipping serum-samples to other places, they MUST be free of the clot, serum separator gel, and red blood cells. It is also important to adhere to the serum collection procedure. For packing, samples MUST be securely contained in a special box with enough dry ice. The package should be appropriately labeled with applicable date, location, tube numbers, and sample type in accordance with international regulations covering the transport of clinical specimens and infectious substances.

4. Handling Precautions

1) The used kits, samples and consumables must be disposed of in accordance with relevant regulations.
2) Be careful not to use an expired kit.
3) Be careful not to use mixed components with different lot numbers.
4) **Since the hemolysis of blood causes misleading results like false positives, it is strongly recommended NOT to use samples (serum) from hemolyzed blood.**
5) Before using this kit, all components must be warmed up to 25°C.
6) New pipette tips must be used for each specimen or standard substance to avoid cross contamination.
7) The level of absorbance is related to reaction time and temperature. Therefore, before starting the test, prepare all reagents, keep the lid open, and have the plate properly prepared so that the elapsed time for all the busy stages is as short as possible.
8) Use all reagents carefully so that they do not make any bubbles.
9) Once the test has started, complete all the steps in one session.
10) Set the pipette to the correct volume.
11) If crystals are found in reagents, dissolve them carefully by gently inverting or vortexing so as not to make any bubbles.
12) Use sterilized tubes, tips, and test containers (15 ㎖, 50 ㎖ tubes).
13) Keep the Stop Solution containing 2 N sulfuric acid from contacting the skin or eyes.

5. Specific Precautions

1) Test results should be regarded as a complementary tool for a professional clinician to make a judgement on patient management.

Serum Collection and Preparation

It is recommended to follow the procedures described below to separate the serum properly.

► Serum collection from a subject

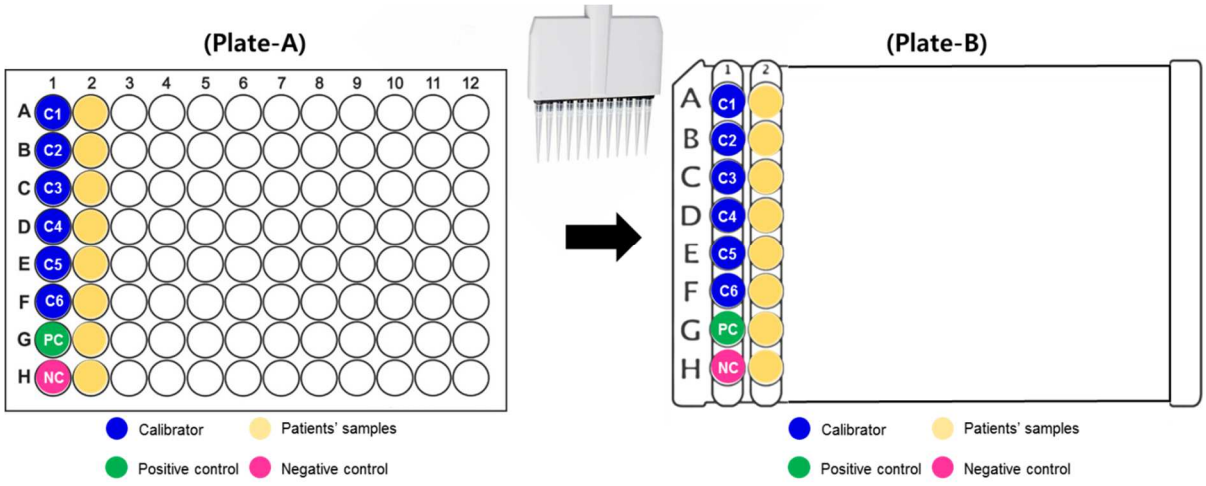
1) Collect blood as described in the serum preparing precautions. Leave the collected at room temperature above 6 hours (for coagulation).
   ☞ Test results are most accurate when serum is separated above 6 hours of collecting blood.
2) Collect the supernatant (serum) test sample after centrifugation for 15 minutes at 3,000 rpm. If the blood is not completely coagulated, or if the subjects are prescribed anticoagulant, coagulation may require more time.
3) Immediately after centrifugation, separate serum samples into 1.5 ㎖ tubes and store at -70°C for long term storage. Avoid repeated freeze-thaw cycles.
4) Thaw frozen serum completely at 2-8°C before test.

Assay Procedure

► General Remarks

1) **ALL components of the kit and samples should be warmed up at 25°C before use.**
2) Be careful not to make bubbles in reagents. When precipitate is observed in the reagents, it should be re-dissolved completely by shaking gently, avoiding bubbles.
3) Once the test is started, complete the whole procedure in one session.
4) When handling test samples, calibrators and reagents, use new sterilized pipette tips in order to avoid cross contamination.
5) As absorbance level of the final reaction mixture is affected by incubation time and temperature, make sure that all reagents, disposables, and plates are ready in order to make the time required for pipetting as short as possible.
6) A standard curve analysis must be carried out simultaneously for each assay.
7) Spin down all tubes temporarily and mix 5 to 10 times by pipetting up and down. Repeat this procedure for each solution.

1. Apply 250 ㎕ each of C1-C6 Calibrator solutions (C6 is blank containing Reagent Dilution Buffer only), Positive Control (PC), and Negative Control (NC) to the appropriate wells in column 1 of **Plate-A** (Uncoated Plate). In the remaining columns, apply 250 ㎕ of each sample (serum). Transfer 100 ㎕ of the contents of each well from **Plate-A** to the corresponding well of **Plate-B** (Antibody Coated 96-well Plate) using a multichannel pipette as quickly as possible.
   ☞ Entire time of solution transfer from **Plate-A** to **Plate-B** should be under 10 minutes to minimize the time deviation of each well.



2. Cover the plate with the plate cover and incubate for 60 (±5) minutes at 20-25°C while gently shaking to ensure thorough mixing. (e.g. 400 rpm)
   ☞ The plate shaker speed was determined using a Thermo Scientific Compact Digital

Microplate Shaker (Cat # 88880023).

3. Prepare diluted Washing Solution (1x) by diluting Washing Solution (10x) using distilled water (DW). Diluted Washing Solution is stable at room temperature for one week. Mix thoroughly and gently.

e.g.	For the preparation of 30 ㎖ of diluted Washing Solution, mix 3 ㎖ of Washing Solution (10x) with 27 ㎖ of DW.

4. Prepare diluted Detection Antibody (1x) by diluting Detection Antibody (1,000x) using Reagent Dilution Buffer. Mix thoroughly and gently.

e.g.	For the preparation of 3 ㎖ (30 tests) of diluted Detection Antibody, mix 0.003 ㎖ (3 ㎕) of Detection Antibody with 2.997 ㎖ of Reagent Dilution Buffer. ☞ Remove 3 ㎕ from 3 ㎖ of Reagent Dilution Buffer.

5. After the reaction is complete, discard the reactant solution from all the wells and remove the remaining solution by tapping the plate against the absorbent paper towels.
   ☞ Do not leave the plate out for a long time after tapping to prevent drying of the wells.
6. Wash each well with 200 ㎕ of the diluted Washing Solution and remove the solution as above. Repeat this process 3 times.
   ☞ Complete removal of solution at each step is essential for good performance.
7. After washing, apply 100 ㎕ of diluted detection antibody solution to each well.
8. Cover the plate and incubate for 120 (±10) minutes at 20-25°C while gently shaking to ensure thorough mixing.
9. After the reaction is completed, remove the solution and wash each well as in Step 5 and 6. (3 times)
10. After washing, apply 100 ㎕ of TMB solution to each well.
    ☞ The color of the solution in the wells should begin to turn blue.
11. Cover the plate and incubate for 10 minutes in a dark place at room temperature (15-25°C).
    ☞ Wrap or cover the plate using aluminum foil.
12. Apply 100 ㎕ of STOP Solution to each well and immediately measure the optical density (OD) of each well using a microplate reader at a wavelength of 450 nm.
    ☞ After adding STOP Solution, the color of the solution in the wells must change completely from blue to yellow.
    ☞ If shaking is possible during the coloration step, it is recommended to terminate the reaction by shaking for 10-15 seconds after adding the STOP Solution.
    ☞ Read the plate within 10 minutes of adding the STOP Solution

Quality Control Procedure

The quality control method for the DxMe® BC Kit assay is analysis of the measured values for the Positive and Negative Control, which are obtained using a plate from each lot that was used. If further quality control procedures are required in your laboratory, follow your laboratory-specific procedures. Ensure that control assay values are within the specific acceptable ranges below.

1) Positive Control (PC) acceptable range: 17.64 ~ 30.56 ng/㎖
2) Negative Control (NC) acceptable range: 3.41 ~ 7.39 ng/㎖
3) $R^2 \geq 0.980$

Limitations of the Procedure

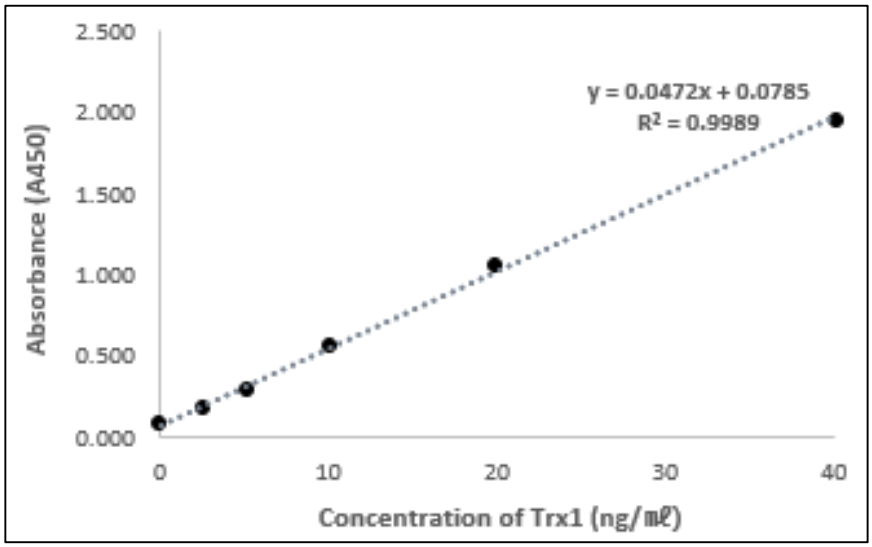
1. Results MUST NOT be used as the sole factor in the diagnosis of breast carcinoma. They should be used by clinical professionals in conjunction with other data, such as symptoms, BI-RADS™, MRI, PET-CT, biopsy, or clinical information.
2. Regardless of Trx1 level, measured results should NOT be interpreted as the sole basis for diagnosis of malignancy.
3. For patients with suspected or diagnosed cancer, other clinical procedures should be considered for proper diagnosis and management.
4. If the results are not consistent with clinical information, it is recommended to perform the test again with corresponding samples. If the same results are obtained through a second test, confirm that there is not a problem with the state of the kit (contamination, storage state, expiration date, etc.).
5. The concentration of Trx1 is elevated in cases of gastric and colorectal cancers. High Trx1 concentrations that are not consistent with clinical and diagnostic charateristics require other clinical diagnostic procedures.
6. Samples which contain concentration of hemoglobin is more than 0.25 g/L may show false positive values, so It is strongly recommended NOT to use samples (serum) from hemolyzed blood.
7. Additional clinical or diagnostic data may be required to determine whether patient status is truly positive or not.

Calculation and Evaluation of Results

1. When it is necessary, calculate the mean value of absorbance from the standard materials and the samples, respectively.
2. A standard curve is created by plotting the absorbance values (vertical axis, Y) versus the known concentrations of the C1-C6 Calibrators (horizontal axis, X), which are from the wells on the reserved column 1 of the plate for the standard curve.
3. Make sure the R^2 value of the standard curve is higher than 0.98 when analyzed by linear regression. This can be done by statistics software such as Excel, MedCalc, or similar statistics programs.

4. The absorbance value of each well containing the testing serum is plotted to the standard curve to calculate the value of the Trx1 concentration for the corresponding well.
5. Make sure the measured values of Positive and Negative Controls are within corresponding acceptable ranges.
6. It is regarded as healthy if the level of Trx1 is below 11.4 ng/㎖.

Example of Standard Curve



Row	Calibrator	Concentration (ng/㎖)	Absorbance (450 nm)
A	C1	40	1.948
B	C2	20	1.062
C	C3	10	0.555
D	C4	5	0.292
E	C5	2.5	0.181
F	C6	0	0.089
G	Positive Control	17.64~30.56	1.052
H	Negative Control	3.41~7.39	0.316

Software and Process for Analysis

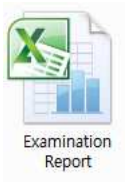
You can download the IFU and the Excel data format files for calculation and evaluation of results from our website (www.ens-h.com).

1. Save the raw data from your test to the 'Raw data form' file that we provided, as shown below. If you already obtained the raw data using the Excel file, you don't need to use this form. (Previous installation of the Excel program is required).

Raw data form (for example)

<>	1	2	3	4	5	6	7	8	9	10	11	12
A	1.9480	0.6748	0.5557	0.7628	0.1337	0.4437	0.8900	0.7006	0.1868	0.3656	0.7615	0.8737
B	1.0620	0.2644	0.4657	0.7312	0.4223	0.9589	0.2314	0.4448	0.5324	0.3994	0.5993	0.6322
C	0.5550	0.4523	0.7214	0.1281	0.7006	0.6016	0.9969	0.1591	0.5809	0.4599	0.8186	0.4827
D	0.2920	0.2026	0.8311	0.7715	0.3365	0.2114	0.1420	0.3102	0.1307	0.6177	0.6361	0.7507
E	0.1810	0.1717	0.2117	0.7181	0.6011	0.3129	0.1585	0.7573	0.8740	0.1013	0.8249	0.5971
F	0.0890	0.9176	0.1023	0.8529	0.8507	0.7561	0.7613	0.2637	0.6063	0.9976	0.1098	0.6481
G	1.0520	0.7469	0.5309	0.6936	0.9911	0.4230	0.7708	0.5347	0.8657	0.4769	0.9127	0.9407
H	0.3160	0.5193	0.6423	0.7380	0.5199	0.1860	0.5911	0.5059	0.7485	0.9031	0.4426	0.3093

2. Double-click the Examination Report (BC) icon to run the analysis software as shown below.



Examination Report(BC)

1. (Experiment Information)

File No.		Date		Tester	
Product Name		Product Lot No.		Reference	DxMe BC KIT
Title					
Purpose	Test for breast cancer				

2. (Materials & Methods)

Materials

3. (Results)

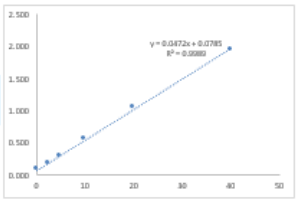
1. You can copy your raw data from EUSA and then paste it in our Report File.

A) Input area

	ng/ml	1	2	3	4	5	6	7	8	9	10	11	12
A	40	1.9480	0.6748	0.5557	0.7628	0.1337	0.4437	0.8900	0.7006	0.1868	0.3656	0.7615	0.8737
B	20	1.0620	0.2644	0.4657	0.7312	0.4223	0.9589	0.2314	0.4448	0.5324	0.3994	0.5993	0.6322
C	10	0.5550	0.4523	0.7214	0.1281	0.7006	0.6016	0.9969	0.1591	0.5809	0.4599	0.8186	0.4827
D	5	0.2920	0.2026	0.8311	0.7715	0.3365	0.2114	0.1420	0.3102	0.1307	0.6177	0.6361	0.7507
E	2.5	0.1810	0.1717	0.2117	0.7181	0.6011	0.3129	0.1585	0.7573	0.8740	0.1013	0.8249	0.5971
F	0	0.0890	0.9176	0.1023	0.8529	0.8507	0.7561	0.7613	0.2637	0.6063	0.9976	0.1098	0.6481
G	PC	1.0520	0.7469	0.5309	0.6936	0.9911	0.4230	0.7708	0.5347	0.8657	0.4769	0.9127	0.9407
H	NC	0.3160	0.5193	0.6423	0.7380	0.5199	0.1860	0.5911	0.5059	0.7485	0.9031	0.4426	0.3093

B) Calculation Area

Conc	Cal				
40	1.948				
20	1.062			INTERCEPT Value	
10	0.555				0.0785
5	0.292			Slope Value	
2.5	0.181				0.0472
0	0.089			R^2	
PC	1.052				0.9989
NC	0.316			positive quantity	20.64
				Negative quantity	5.03



2. Compare it with our Acceptance Range.

C) Result Area

Acceptance Range		our DATA
R^2	≥0.98	0.9989
Positive Control (PC) acceptable range	17.64~30.56 ng/ml	20.64
Negative Control (NC) acceptable range	3.41~7.39 ng/ml	5.03

D) patient result area — Patients samples turn to red(Over cut-off value) — <Cut-off value : 11.4 ng/ml>

	ng/ml	1	2	3	4	5	6	7	8	9	10	11	12
A	40	Cal	12.64	10.11	14.51	1.17	7.74	17.20	13.19	2.30	6.09	14.48	16.86
B	20	Cal	3.94	8.21	13.84	7.29	18.66	3.24	7.77	9.62	6.80	11.04	11.74
C	10	Cal	7.92	13.63	1.05	13.19	11.09	19.47	1.71	10.65	8.09	15.69	8.57
D	5	Cal	2.63	15.95	14.69	5.47	2.82	1.35	4.91	1.11	11.43	11.82	14.25
E	2.5	Cal	1.98	2.82	13.56	11.08	4.97	1.69	14.39	16.86	0.48	15.82	10.99
F	0	Cal	17.79	0.50	16.42	16.37	14.36	14.47	3.93	11.19	19.48	0.66	12.08
G	PC	PC	14.17	9.59	13.04	19.35	7.30	14.67	9.67	16.69	8.44	17.68	18.28
H	NC	NC	9.34	11.95	13.98	9.36	2.28	10.87	9.06	14.20	17.48	7.72	4.89

Mean : ≥ Cut-off Mean : < Cut-off

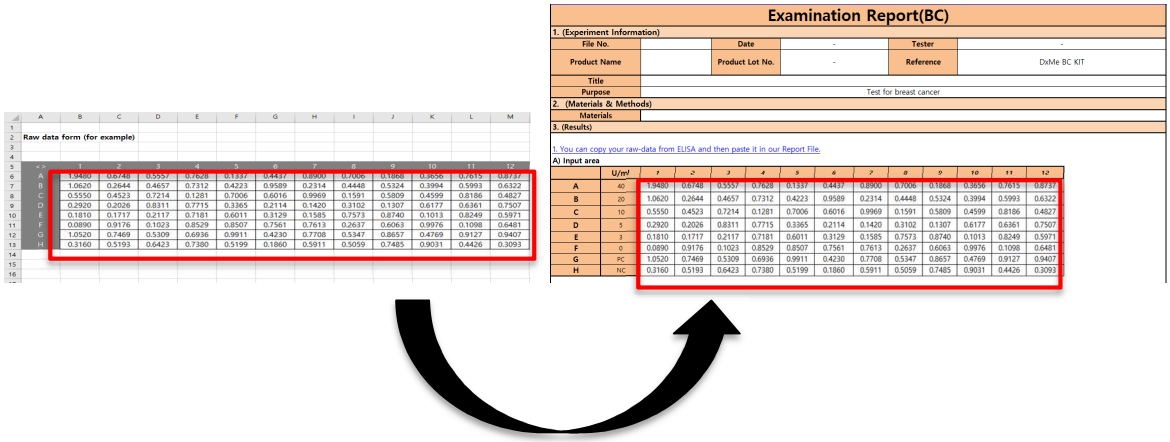
A) Input Area: place to paste the raw-data

B) Calculation Area: show the calculated and analyzed standard

C) Result Area: show the result for comparing with the acceptable ranges

D) Patient's Result Area: show the results of the patient's serum

3. Copy your raw data and paste them to A) Input Area of Examination Report.

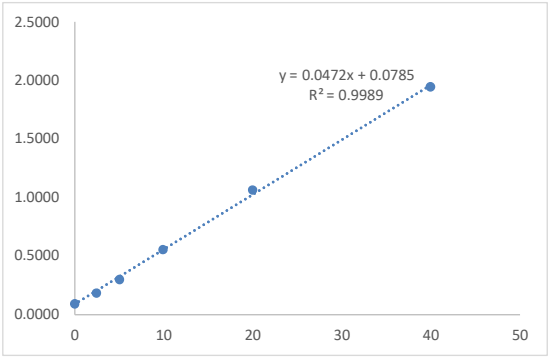


Tip) If you cannot understand this procedure, click the hyper-link in Examination Report (1. You can copy your raw-data from ELISA test and then paste them to our pre-set form). It shows the place to paste the raw data.

4. The automatically analyzed results will be shown as below in B) Standard Calculation Area.

B) Calculation Area

Conc	Cal				
40	1.9480				
20	1.0620			INTERCEPT Value	
10	0.5550				0.0785
5	0.2920			Slope Value	
2.5	0.1810				0.0472
0	0.0890			R^2	
PC	1.0520				0.9989
NC	0.3160		positive quantity		20.64
			Negative quantity		5.03



5. If you look at the C) Standard Result Area, you can compare your results with acceptance criteria. When the results are within the acceptable range, you can judge the risk of breast cancer. It can be viewed by clicking the hyper-link (2. Compare it with our Acceptable Range).

☞ Make sure the measured values of Positive and Negative Controls are within the corresponding acceptable ranges. If one or both value(s) is(are) out of the ranges, the test is regarded as a fail.

6. If you look at the D) Patient Result Area, you can also check the results of the patient's serum. When the result is over the cut-off value (11.4 ng/㎖), it will turn red.

D) patient result area — Patients samples turn to red(Over cut-off value) — <Cut-off value : 11.4 ng/ml>

	ng/ml	1	2	3	4	5	6	7	8	9	10	11	12
A	40	Cal	12.64	10.11	14.51	1.17	7.74	17.20	13.19	2.30	6.09	14.48	16.86
B	20	Cal	3.94	8.21	13.84	7.29	18.66	3.24	7.77	9.62	6.80	11.04	11.74
C	10	Cal	7.92	13.63	1.05	13.19	11.09	19.47	1.71	10.65	8.09	15.69	8.57
D	5	Cal	2.63	15.95	14.69	5.47	2.82	1.35	4.91	1.11	11.43	11.82	14.25
E	2.5	Cal	1.98	2.82	13.56	11.08	4.97	1.69	14.39	16.86	0.48	15.82	10.99
F	0	Cal	17.79	0.50	16.42	16.37	14.36	14.47	3.93	11.19	19.48	0.66	12.08
G	PC	PC	14.17	9.59	13.04	19.35	7.30	14.67	9.67	16.69	8.44	17.68	18.28
H	NC	NC	9.34	11.95	13.98	9.36	2.28	10.87	9.06	14.20	17.48	7.72	4.89

Mean : ≥ Cut-off Mean : < Cut-off

Clinical Performance Characteristics

1. The normal distribution of Trx1 levels determined by the DxMe® BC Kit
   1) The apparently healthy person's 336 samples are used to determine normal range of Trx1.
   2) The apparently healthy samples originated from Chungnam National University Hospital and were analyzed in pivotal clinical trials with IRB approval.

The Normal Distribution of DxMe® BC kit assay values					
Apparently Healthy	Number of subjects	0 - 5 ng/㎖	5 – 11.4 ng/㎖	11.4 - 15 ng/㎖	15 - 20 ng/㎖
Females	336	105	222	6	3
Percentage(%)	100.00	31.25	66.07	1.79	0.89

In the study, 97.32% of the apparently healthy females had lower than the cut-off value, 11.4 ng/㎖. Furthermore, the test values of the DxMe® BC kit were not correlated to menopause status, age, stage, histopathological grade, hormone receptors' status, or Ki67 expression, so the results from the DxMe® BC tests are not influenced by the physiological and pathological situations described above[10-11].

2. Reference Interval of the DxMe® BC kit
   1) Reference intervals were analyzed in compliance with CLSI C28-A3 guidelines.
   2) There were higher than 120 samples tested by DxMe® BC Kit, so the analysis uses a non-parametric percentile method.

The Reference Interval of DxMe® BC kit assay values (ng/ml)						
Sample size	Lowest value	Highest Value	Mean	Median	Lower limit	Upper limit
336	0.23	20.58	6.62	6.39	1.87	11.83

3. DxMe® BC kit's ability to diagnose breast cancer
   1) A pivotal clinical study was conducted for breast cancer patients and women without breast cancer to demonstrate the effectiveness of supporting breast cancer diagnosis.
   2) A total of 644 samples were used, analyzed, and an ROC analysis was performed.

ROC analysis of DxMe® BC kit assay						
Sample size	Sensitivity	Specificity	AUC±SE	95% CI	Cut-off	P-value
644	96.43%	97.32%	0.985±0.005	0.972 to 0.993	11.4ng/ml	<0.001

Analytical Performance Characteristics

Analytic sensitivity	Limit of blank	0.344 ng/mL The Limit of Blank(LoB) was evaluated according to CLSI guideline EP17-A2 by testing 4 analyte-free samples. Samples were tested 4 replicates each run in 4 runs using 2 different lots to reach a total of 64 measurements per lot. The LoB was determined to be 0.344 ng/mL using the non-parametric analysis.
	Limit of Detection	0.649 ng/mL The Limit of Detection(LoD) was evaluated according to CLSI guideline EP17-A2 by testing 4 low concentration human serum pools samples. Samples were tested 4 replicates each run in 4 runs using 2 different lots to reach a total of 64 measurements per lot. The LoD was determined to be 0.649 ng/mL using the parametric analysis.
	Limit of Quantitation	1.474 ng/mL The Limit of Quantitation(LoQ) was evaluated according to CLSI guideline EP17-A2 and EP15-A3 by testing 4 low concentration human serum pools samples. Samples were tested 5 replicates each run in 5 runs using 2 different lots to obtain 100 measurements per lot. The LoQ was determined to be 1.474 ng/mL that concentration show the within-Laboratory CV within 10%. Additionally, the total error percentage(%TE) for 1.474 ng/mL was 26.1%.
	Measurement range	1.47~41.42 ng/mL The measurement range was evaluated according to CLSI guideline EP06-A2 by testing samples made by diluting 2 human serum pools to 9 concentrations each. Samples were tested 7 replicates using 2 different lots and the measurement range was determined to be 1.47~41.42 ng/mL.
	Cut-off value	11.4 ng/mL A retrospective exploratory clinical trial was conducted for breast cancer patients and normal peoples. As a result of ROC analysis through 114 breast cancer patient samples and 106 normal samples, Cut-off value were determined 11.4 ng/mL.

<table>
<tr><td rowspan="2">Analytical Specificity</td><td>Cross-reactivity</td><td>The cross-reactivity test was performed in accordance with CLSI guidelines EP07-A3 and EP37-A by testing human serum at two concentrations, high and low.
No interference with 14 cross-reaction substances was observed at the stated concentration.

- Cross-reaction substance: AFP (60ng/mL), β-hCG (150mIU/mL), BRCA 1 (100ng/mL), BRCA 2 (100ng/mL), CA125 (50U/mL), CA15-3 (50U/mL), CA19-9 (50U/mL), CEA (15ng/mL), C-myc (100ng/mL), HER2 (100ng/mL), HSP60 (100ng/mL), Prx1 (50U/mL), PSA (30ng/mL), Trx2 (50U/mL)</td></tr>
<tr><td>Interference</td><td>The interference test was performed in accordance with CLSI guidelines EP07-A3 and EP37-A by testing human serum at two concentrations, high and low.
No interference with 39 substance were observed at the stated concentration.

- Endogenous substance (Total 7)
Bilirubin, Conjugated (2mg/dL), Cholesterol, total (220mg/dL), HAMA (100.9 ng/mL), Hemoglobin(High serum pool: ~0.75g/L, Low serum pool: ~0.25g/L), Human albumin (52g/L), Rheumatoid factor (2085 IU/mL) Triglycerides, total (250mg/dL)

- Exogenous substance (Total 32)
5-fluorouracil (9mg/dL), Acetaminophen (15.6mg/dL), Acetylcysteine (15mg/dL), Acetylsalicyclic acid (3mg/dL), Ampicillin (7.5mg/dL), Ascorbic acid (5.25 mg/dL), Atezolizumab (720mg/L), Biotin (0.351 mg/dL), Carboplatin (566 mg/L), Cefoxitin (660 mg/dL), Celecoxib (0.879 mg/dL), Cyclophsphamide (54.9 mg/dL), Cyclosporine (0.18 mg/dL), Diclofenac sodium (2.4 mg/dL), Docetaxel (142 mg/L), Doxorubicin (144mg/L), Doxycycline (1.8 mg/dL), Etoposide (142 mg/L), Heparin (330 units/dL), Ibuprofen (21.9mg/dL), Levodopa (0.75mg/dL), Megestrol (192 mg/L), Methotrexate (136 mg/dL), Methyldopa (2.25 mg/dL), Metronidazole (12.3 mg/dL), Mitomycin (18 mg/L), Rifampicin (4.8 mg/dL), Tamoxifen (0.0519mg/dL), Taxol (425mg/L), Theophylline (6mg/dL), Trastuzumab (480 mg/L), β-estradiol (7.5 pg/mL)</td></tr>
<tr><td>Precision</td><td>Repeatability</td><td>The repeatability was evaluated according to CLSI guideline EP05-A3 by testing 4 human serum pool samples and 2 control samples (PC and NC). Samples were tested in 4 replicates per run, 2 runs per day, for 20 days using one reagent lot. The result are summarized in the follwing table.
<table>
<tr><th rowspan="2">Sample</th><th rowspan="2">Mean (ng/mL)</th><th colspan="2">Repeatability</th><th colspan="2">Within-Laboratory</th></tr>
<tr><th>SD</th><th>%CV</th><th>SD</th><th>%CV</th></tr>
<tr><td>Sample 1</td><td>20.51</td><td>0.74</td><td>3.6%</td><td>1.05</td><td>5.1%</td></tr>
<tr><td>Sample 2</td><td>10.23</td><td>0.49</td><td>4.8%</td><td>0.61</td><td>6.0%</td></tr>
<tr><td>Sample 3</td><td>4.53</td><td>0.19</td><td>4.3%</td><td>0.40</td><td>8.9%</td></tr>
<tr><td>Sample 4</td><td>1.48</td><td>0.07</td><td>4.4%</td><td>0.14</td><td>9.5%</td></tr>
<tr><td>PC</td><td>19.80</td><td>0.49</td><td>2.5%</td><td>1.02</td><td>5.1%</td></tr>
<tr><td>NC</td><td>4.55</td><td>0.24</td><td>5.2%</td><td>0.34</td><td>7.5%</td></tr>
</table></td></tr>
</table>

Reproducibility

The reproducibility was evaluated according to CLSI guideline EP05-A3 by testing 4 human serum pool samples and 2 control samples (PC and NC). Samples were tested in 4 replicates per run, 2 runs per day, for 5 days with 3 conditions(Lots, Operators, Sites). The result are summarized in the follwing table.

1) Lot-to-Lot reproducibility
Lot-to-Lot reproducibility was tested for 5 days using 3 lots of kit.

Sample	Mean (ng/mL)	Repeatability		Within-Lot		Within-Laboratory	
		SD	%CV	SD	%CV	SD	%CV
Sample 1	21.16	0.72	3.4%	0.80	3.8%	0.97	4.6%
Sample 2	10.29	0.50	4.8%	0.60	5.8%	0.60	5.8%
Sample 3	5.00	0.22	4.5%	0.36	7.2%	0.38	7.6%
Sample 4	1.62	0.07	4.6%	0.15	9.0%	0.15	9.3%
PC	20.19	0.48	2.4%	0.72	3.5%	0.84	4.2%
NC	4.61	0.21	4.5%	0.25	5.5%	0.26	5.6%

2) Operator-to-Operator reproducibility
Operator-to-Operator reproducibility was tested by 3 operators for 5 days.

Sample	Mean (ng/mL)	Repeatability		Within-Operator		Within-Laboratory	
		SD	%CV	SD	%CV	SD	%CV
Sample 1	21.27	0.71	3.3	0.93	4.4	0.96	4.5
Sample 2	10.28	0.53	5.2	0.72	7.0	0.72	7.0
Sample 3	5.11	0.23	4.5	0.37	7.1	0.43	8.5
Sample 4	1.59	0.09	5.6	0.15	9.1	0.15	9.5
PC	20.28	0.59	2.9	0.78	3.8	0.83	4.1
NC	4.56	0.17	3.7	0.22	4.9	0.24	5.3

2) Site-to-Site reproducibility
Site-to-Site reproducibility was tested at 3 sites for 5 days.

Sample	Mean (ng/mL)	Repeatability		Within-Site		Within-Laboratory	
		SD	%CV	SD	%CV	SD	%CV
Sample 1	21.08	0.74	3.5%	0.95	4.5%	0.95	4.5%
Sample 2	10.37	0.60	5.8%	0.76	7.3%	0.76	7.3%
Sample 3	5.24	0.20	3.8%	0.42	7.9%	0.47	8.9%
Sample 4	1.57	0.08	5.1%	0.14	8.8%	0.14	8.8%
PC	19.95	0.61	3.1%	0.80	4.0%	0.80	4.0%
NC	4.50	0.18	4.0%	0.27	5.9%	0.27	5.9%

Accuracy

The accuracy was performed in accordance with CLSI guideline EP15-A3. The accuracy was evaluated by testing with 3 samples, dilution reagent, artificial serum, and human serum. These samples were spiked with Trx1 protein. Spiked samples were tested 5 replicates each run in 5 runs.
Through Precision and Bias analysis, it was determined that the relative bias(%Bias) was within ±10% in all samples.

Troubleshooting

Problem: Weak or no signal in ELISA	
Possible Cause	**Solution**
Reagents not at room temperature at the start of assay	It is recommended that all reagents be at room temperature before starting the assay. Allow reagents to sit on bench or incubator for enough time (e.g., 120 minutes) to reach room temperature (25 °C).
Incorrect storage of components	Double check storage conditions indicated on label of the kit. DxMe® BC kit needs to be stored at 2–8°C.
Expired reagents	Confirm expiration dates of all reagents. Do not use reagents that are past the expiration date.
Reagents added/prepared incorrectly	Check protocol, ensure correct volume of each reagent was added or mixed in the proper order to prepare the correct concentration.
Incorrect dilutions prepared	Check pipetting technique and double check calculations. It is recommended that all testers are certified by proficiency test.
Wells scratched with pipette or washing tips	Use caution when dispensing and aspirating into and out of wells. Automated plate washers may need to be calibrated so that tips don't touch the bottom of wells.
Plate read at incorrect wavelength	Double check wavelength setting. Ensure plate reader is set accurately for type of substrate being used.
Incubation time with shaking too short	Check protocol to follow exact procedure. It is recommended that all testers are certified by proficiency test.
Precipitate can form in wells upon substrate addition when concentration of target is too high	Carefully check the procedure for correct dilution of reagent before starting test. Carry out dilution of sample and re-try the experiment.
Using incompatible sample type (e.g. serum vs. cell extract)	Detection may be reduced or absent in untested sample types. Double check sample type.
Sample prepared incorrectly	Ensure proper sample preparation described in instructions for use.

Insufficient washing	Use appropriate washing procedure from IFU. At the end of each washing step, invert plate on absorbent tissue or paper towel and allow to completely drain, tapping forcefully if necessary to remove any residual fluid.
Plate covers not used	During incubations, covering the plate will prevent wells from contaminating each other by spattering.
Incorrect dilutions prepared	Check pipetting technique and double-check calculations. It is recommended that all testers are certified by proficiency test.
Longer incubation times than recommended	Check assay procedure again. It is recommended that all testers are certified by proficiency test.

Problem: High background in ELISA	
Possible Cause	**Solution**
Insufficient washing	Use appropriate washing procedure. Increasing duration of soaking steps may also help. At the end of each washing step, invert plate on absorbent tissue and allow to completely drain, tapping forcefully if necessary to remove any residual fluid.
TMB substrate exposed to light prior to use	Ensure TMB substrate is not exposed to light and is stored in a dark place. Limit exposure to light while running assay.
Longer incubation times than recommended	Check assay procedure again. It is recommended that all testers are certified by proficiency test.
Incorrect standard curve dilutions prepared	Check pipetting technique and double-check calculations. It is recommended that all testers are certified by proficiency test.
Contaminated washing solution	Check if washing solution is transparent or not. If it is not clear, do not use it. Make fresh wash buffer and use clean bottle.
Waiting too long to read plate after adding STOP solution	Read plate immediately, within 10 min of adding STOP solution.

Problem: Poor standard curve in ELISA	
Possible Cause	**Solution**
Standard improperly prepared	Briefly spin vial before opening; thoroughly re-suspend if crystal is found in the solution powder. Make sure to pipette correct amount of Standard Solution.
Standard degraded	Store sample as recommended.
Curve doesn't fit scale	Try plotting using different scale.

Problem: Poor replicate data in ELISA	
Possible Cause	**Solution**
Insufficient washing	Use appropriate washing procedure. Increasing duration of soaking steps may also help. At the end of each washing step, invert plate on absorbent tissue and allow to completely drain, tapping forcefully if necessary to remove any residual fluid.
Plate covers not used	During incubations, covering the plate will prevent wells from contaminating each other by spattering.
Bubbles in wells	Ensure no bubbles are present prior to reading plate or set the microplate reader to shake the plate for 5 seconds.
All wells not washed equally/thoroughly	Check that all ports of plate washer are unobstructed. Wash wells as recommended.
Incomplete reagent mixing	Ensure all reagents/master mixes are mixed thoroughly.
Inconsistent pipetting	Use calibrated pipettes and ensure accurate pipetting.
Inconsistent sample preparation or storage	Ensure consistent sample preparation and optimal sample storage conditions (e.g. restrict repeated freeze-and-thaw cycle of samples)

Problem: Inconsistent results test-to-test in ELISA	
Possible Cause	**Solution**
Insufficient washing	Use appropriate washing procedure. Increasing duration of soaking steps may also help. At the end of each washing step, invert plate on absorbent tissue and allow to completely drain, tapping forcefully if necessary to remove any residual fluid.
Inconsistent incubation temperature	Make sure to follow recommended incubation temperatures in IFU. Be aware of fluctuations in temperature due to environmental conditions.
Plate covers not used	During incubations, covering the plate will prevent wells from contaminating each other by spattering.
Incorrect dilutions prepared	Check pipetting technique and double-check calculations. It is recommended that all testers are certified by proficiency test.

Problem: Edge effects in ELISA	
Possible Cause	**Solution**
Uneven temperature	Before starting to test using the DxMe® BC kit, carry out validation and verification tests of temperature at the edge point of incubator. Make sure plate is placed in the center of calibrated and validated incubator.
Evaporation	It is recommended to change the plate cover to a proper sealer to seal the plate completely during incubations.
Stacked plates	Avoid stacking plates during incubation.

Tips on pipetting and washing techniques for ELISA

Pipetting technique

1. Use the correct pipette that is within the range suggested by manufacturer.
2. Confirm tip is firmly seated on the pipette.
3. Confirm there are no air bubbles while pipetting.
4. Change tips between each standard, sample, or reagent.
5. Use different reservoirs for each reagent.
6. Pipette sample into the side of wells to avoid splashing.
7. Always run samples/standards in replicate.

Washing procedure

1. Completely aspirate liquid from all wells by gently lowering an aspiration tip into the bottom of each well or pouring finished reaction fluid into waste and inverting plate on absorbent tissue. Allow to completely drain, tapping forcefully if necessary to remove any residual fluid. Note: Take care not to scratch the inside of the well or touch the bottom.
2. Fill the wells with at least 200 μℓ of diluted wash buffer.
3. Let soak for 15 to 30 seconds if using the aspirating system to wash.
4. Aspirate wash buffer completely from wells.
5. Repeat as directed in protocol (3 times).
6. After washing is completed, invert plate and tap (forcefully, if necessary) on dry absorbent tissue. Be sure to remove any residual liquid.
7. Alternatively, an automated plate washer may be used. Be sure to follow the steps above.

Disposal of the Kit

The used kits, sample sera, and disposables should be discarded according to corresponding disposal procedures. Disposal of all waste material should be in accordance with local guidelines.

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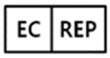

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Version and Revision Date

Version: ESH-IFU-BCE-003-R2en

Revision Date: Jan. 10th. 2022.

Vigilance Notice

If any adverse event involving the DxMe® BC Kit occurs, please notify the Authorized Representative.