Human MAGEA4 ELISA Kit

Catalog No. EA200022

Principle of the Assay

Human melanoma-associated antigen 4 (MAGEA4) is a protein encoded by MAGEA4 gene. This gene is a member of the MAGEA gene family. The members of this gene family encode proteins with 50 to 80% sequence identity to each other. MAGEA4 is highly expressed in a variety of cancers such as melanoma, bladder, breast, head and neck, oral, liver and lung cancers, but not in normal tissues except for testes and placenta. There is significant correlation between MAGEA4 protein expression and tumor invasion.

This sandwich ELISA is used to measure human MAGEA4 in serum, plasma, and other biological fluid samples. Microtitration wells coated with anti-human MAGEA4 capture antibody are exposed to test specimens. The MAGEA4 antigen in the specimen is specifically captured onto the immobilized antibody during specimen incubation. The captured MAGEA4 antigen is then reacted with a biotinylated human MAGEA4 detection antibody. Subsequently, Streptavidin-HRP conjugate is then added. After wash, specifically bound enzyme conjugate is detected by reaction with the Substrate Solution, tetramethylbenzidine (TMB). The assay is measured spectrophotometrically to indicate the level of MAGEA4 present in a sample.

Materials Supplied

Description	Quantity
MAGEA4 Antibody Coated 96-well Plate in foil pouch with desiccant	1
Human MAGEA4 Standard (400ng/mL)	100 µL
Biotinylated MAGEA4 Detection Antibody (100x)	120 µL
Streptavidin Conjugated Horseradish Peroxidase (100x)	120 µL
Assay Buffer	30 mL
Standard Diluent	10 mL
Sample Diluent 1	15 mL
Sample Diluent 2	15 mL
Substrate Solution (TMB)	12 mL
Stop Solution (1N HCl)	12 mL
Wash Buffer (20x)	60 mL
Plate Sealer	3

Additional Materials not Supplied

1. Horizontal orbital plate shaker capable of maintaining a speed of 450±50 rpm.
2. Disposable tip micropipettes to deliver volumes of 5µL to 200 µL (multichannel pipette preferred for dispensing reagents into microtiter plates).
3. Distilled or deionized water.
4. Clean, disposable plastic/glass test tubes, approximate capacities 5mL and 15mL.
5. Laboratory glassware consisting of 100 mL beaker, 100 mL and 1 L graduated cylinders, 5 mL, 10mL and 25 mL pipettes.
6. Absorbent paper towels.
7. Automatic microplate washer or laboratory wash bottle.
8. Microplate reader with 450nm filter.
9. Latex gloves, safety glasses and other appropriate protective garments.
10. Biohazard waste containers.
11. Safety pipetting devices for 1 mL or larger pipettes.
12. Timer.

Storage and Stability

Upon receipt, store the kit at 2-8°C. The kit should not be used beyond the expiration date. Once opened, the unused microplate strips should be returned to their original foil pouch along with the desiccant. The diluted Wash Buffer should not be stored for longer than 3 weeks at 2-8°C. It is recommended that Wash buffer be freshly diluted before each assay. If the diluted Wash buffer becomes visibly cloudy during the 3 weeks, discard it. (Note: Concentrated Wash Buffer, when stored at 2-8°C, normally may develop crystalline precipitates, which can be re-dissolved at 37°C.)

Indications of Deterioration

The human MAGEA4 Assay kit may be considered to have deteriorated if:

1. Reagents are visibly cloudy.
2. The Substrate Solution turns blue. This is likely to be caused by chemical contamination of the Substrate Solution.

Precautions

1. The reagents supplied in this kit are for ***Research use only***.

2. All blood products should be treated as potentially infectious.

3. Disposal or decontamination of fluid in the waste reservoir should be in accordance with guidelines described in the Department of Labor, Occupational Safety and Health Administration, occupational exposure to blood-borne pathogens; final rule (29 CFR 1910,1030) FEDERAL REGISTER, pp. 64176-84177,12/6/91.

4. The Substrate Solution and Stop Solution in this kit can irritate the skin and cause eye damage. Handle them with care and wear protective gloves, clothing and eye/face protection. Wash hands thoroughly after handling. Immediately flush the affected area with plenty of water in case of contact with skin or eyes. Obtain medical attention if necessary.

Technical Suggestions

1. This kit should be used in strict accordance with the instructions in the Package Insert.
2. Do not use the kit after the expiration date printed on the outer carton label.
3. Do not cross contaminate reagents.

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4. Some reagents in the MAGEA4 ELISA kit are optimized for each kit lot. Do not exchange reagents from kits with different lot numbers.
5. To ensure accurate results and avoid cross-contamination, use proper adhesive plate sealers during incubation steps, and change pipette tips when adding each standard and sample. Multi-channel pipettes are recommended for large assays. Always use fresh pipette tips when drawing from stock reagent bottles.
6. Warm up the foil bag to room temperature before opening.
7. All reagents should be added to the plate in the same order.
8. If the Stop Solution does not mix thoroughly with the Substrate Solution, the color in the wells may appear green after adding stop solution. Gently tap the plate or pipette up and down to mix until the color in the wells change to yellow (avoid bubbles during this step).
9. Reagents should be dispensed with the tip of the micropipettes touching the side of the well at a point about mid-section. For automatic processors, follow manufacturer's recommendations.
10. It is recommended that all pipetting devices (manual or automatic), and thermometers are regularly calibrated according to the manufacturer's instructions.

Sample Collection and Storage

The Human MAGEA4 ELISA is intended for use with cell culture supernatants, serum, plasma and other biological fluids. The sample should be tested as soon as possible. However, if the sample needs storage, it should be stored frozen at -20°C or below. Do not use self-defrosting freezers. Frozen samples that have been thawed should be thoroughly mixed before testing.

Cell Culture Supernatant: Remove particulates by centrifugation and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.
Serum: Use a serum separator tube (SST) and allow samples to clot for 30 minutes at room temperature before centrifugation for 15 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at ≤ -20 ℃. Avoid repeated freeze-thaw cycles.
Plasma: Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Rinse Cycle

Aspirate each well and wash. Wash by filling each well with Wash Buffer (300µL) using a squirt bottle, manifold dispenser, or automatic plate washer. Complete removal of liquid at each step is essential to good performance. After the last wash, invert the plate and blot it against clean paper towels.

Preparation for the Assay

1. *Standard preparation*: Prepare protein standard 1 by diluting 10µL of standard stock into 490 µL (1:50 dilution) of **Standard Diluent**. This will give a final concentration of 8000 pg/mL as shown in Table 1. Make 2x serial dilution of Standard 1 using sample diluent to generate a standard concentration range of 125 to 8000 pg/mL.

Table 1: Human MAGEA4 Standard Curve Generation

Standard Number	Concentration of MAGEA4 (pg/mL)	MAGEA4 Standard (µL)	Standard Diluent (µL)
1	8000	10	490
2	4000	250 of #1	250
3	2000	250 of #2	250
4	1000	250 of #3	250
5	500	250 of #4	250
6	250	250 of #5	250
7	125	250 of #6	250
8	0		250

2. *Sample preparation*: MAGEA4 concentration must be estimated prior to performing the full experiment by testing a serially diluted representative sample using **Sample Diluent 1** (for serum and EDTA-plasma sample) **or Sample Diluent 2** (for Heparin-plasma and other biological fluids). Select an optimal dilution level such that the final target protein concentration falls near the middle of the assay linear dynamic range. For normal serum and plasma samples, a starting 5-fold dilution is recommended. If sample need more than 200-fold dilution, two or more dilution steps are suggested.

3. *Detection antibody preparation:* dilute the concentrated biotin conjugated detection antibody 1:100 using **Assay Buffer**.

4. *SA-HRP preparation*: dilute the concentrated streptavidin HRP conjugate 1:100 using **Assay Buffer**.

5. *Wash buffer*: Dilute 20x Wash Buffer Concentrate to 1x with distilled or de-ionized water. If a kit is likely to be utilized over a period in excess of 4 weeks, then it is recommended that only enough stock concentrate be diluted sufficient for immediate needs.

Assay Procedure

Note: All standards, controls and samples should be tested in duplicate.

1. Warm up kit to room temperature (18-25°C).

2. Select sufficient microplate strips to accommodate all test samples, controls and reagent blank. Fit the strips into the holding frame.

3. Wash plate two times, add 100 µL of each standard and sample into appropriate wells. Note: Depending on the MAGEA4 concentration of your sample, dilution using sample diluent may be needed.

4. Incubate for 2 hours at room temperature with moderate shaking (450±50rpm) on a horizontal orbital plate shaker.

5. Wash the microtitration plate 3 times as described in the Rinse Cycle section.

6. Add 100 µL of working concentration detection antibody into each well and incubate for 1.5 hour at room temperature with moderate shaking (450±50rpm) on a horizontal orbital plate shaker.

7. Wash the microtitration plate 3 times as described in the Rinse Cycle section.

8. Add 100 µL of working concentration Streptavidin HRP conjugate into each well and incubate for 25 minutes at room temperature with moderate shaking (450±50rpm) on a horizontal orbital plate shaker.

9. Wash the microtitration plate 5 times as described in the Rinse Cycle section.

10. Add 100 µL Substrate Solution into each well. A multichannel pipette should be used for best results. Leave at room temperature (18-25°C) and protected from direct sunlight for 20 minutes.

11. Add 100 µL of Stop Solution to each well. The color in the wells should change from blue to yellow. If the color in the wells is green or the color change does not appear uniform, gently tap the plate to ensure thorough mixing. Ensure that the undersides of the wells are dry and that there are no air bubbles in the well contents.

12. Read the absorbance values at 450 nm using a microplate reader. If wavelength correction is available, set to 540 nm or 570 nm.

Calculation of Results

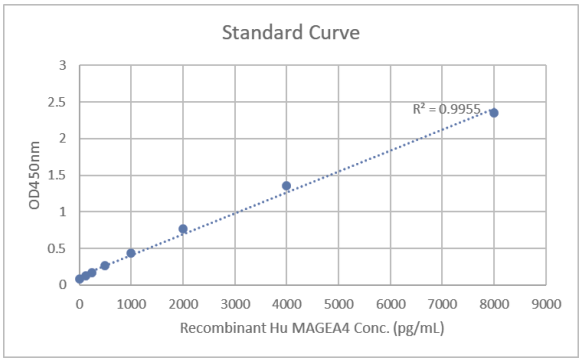
Average the duplicate readings for each standard and sample. A 4-parameter logistic (4-PL) or a linear regression model providing a point-to-point curve fitting provides acceptable results. Create a standard curve by reducing the data using computer software capable of generating a four-parameter logistic (4-PL) or a linear regression curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph. Do not force the line to be linear. The concentration of the samples can be found directly from the standard curve.

Table 2. Example Data at 450nm.

Standards	450 nm absorbance
Standard 1 (8000 pg/mL)	2.35455
Standard 2 (4000 pg/mL)	1.35155
Standard 3 (2000 pg/mL)	0.7669
Standard 4 (1000 pg/mL)	0.4312
Standard 5 (500 pg/mL)	0.2593
Standard 6 (250 pg/mL)	0.1673
Standard 7 (125 pg/mL)	0.1236
Standard 8 (0 ng/mL)	0.07785

Typical Human MAGEA4 ELISA Kit Standard Curve

This standard curve was generated at OriGene for demonstration purpose only. A standard curve must be run with each assay.



Note: This standard curve is only an example and should not be used to generate any results.

Performance Characteristics

1. **Recovery**
   The recovery of human MAGEA4 spiked to three different-levels of the assay range in diluted samples was evaluated

Sample	Average Recovery	Range
Hu serum	96%	89-103%
Hu EDTA Plasma	92%	91-93%
Hu Heparin Plasma	91%	89-92%
Culture Media	97%	96.97-97.38%

2. **Linearity**
   To assess the linearity of the assay, human MAGEA4 spiked samples were diluted to produce samples with values within the dynamic range of the assay.

	Cell culture media	EDTA-plasma	Heparin-plasma	Serum
%Expected at 1:2 dilution	98	102	100	102
%Expected at 1:4 dilution	98	102	97	102
%Expected at 1:8 dilution	99	98	94	95

3. **Sensitivity:** 33.5pg/mL

4. **Precision**
   Human serum, plasma and culture media samples with different levels of MAGEA4 were assayed 10 times each on three different assays. The intra-assay CV percentage and inter-assay CV percentage were calculated.

   Intra-assay:

Sample	%CV in Assay 1	%CV in Assay 2	%CV in Assay 3	Ave %CV
Serum (n=10)	2.00	2.39	3.34	2.58
EDTA-Plasma (n=10)	2.44	2.95	2.05	2.48
Heparin-Plasma (n=10)	3.21	2.15	6.02	3.79
Culture Media (n=10)	5.36	2.22	2.03	3.20

   Inter-assay

Sample	Mean (pg/ml) assay1	Mean (pg/ml) assay2	Mean (pg/ml) assay3	Ave (pg/ml)	SD	%CV
Serum (n=10)	333.08	330.69	366.23	343.33	19.86	5.78
EDTA-Plasma (n=10)	374.01	367.28	399.75	380.35	17.14	4.51
Heparin-Plasma (n=10)	421.79	412.40	436.26	423.48	12.02	2.84
Culture Media (n=10)	482.22	449.38	448.63	460.08	19.18	4.17

Limitations of Use

1. This kit is for research use only. **Not for use in diagnostic procedures.**

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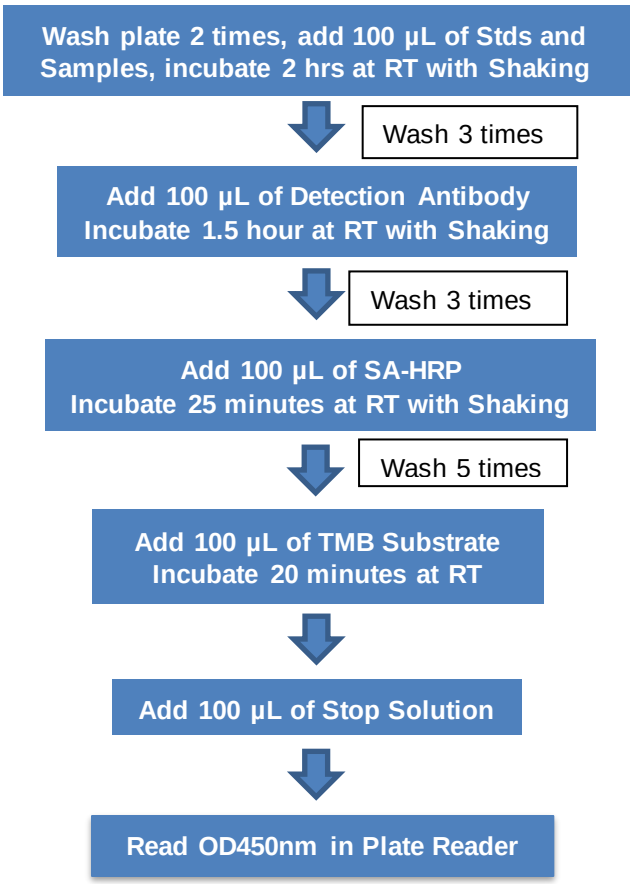
2. The MAGEA4 value measured using OriGene MAGEA4 ELISA kit may not be interchangeable with that obtained from other assay kits.
3. The assay cannot be used to quantitate samples with MAGEA4 values higher than the highest standard without further dilution of the samples.

Assay Flowchart



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