ELISA Kit for Measuring Human WFDC2/HE4

CircuLex Human WFDC2/HE4 ELISA Kit

Cat# CY-8211

Intended Use

The MBL Research Product **CircuLex Human WFDC2/HE4 ELISA Kit** is used for the quantitative measurement of human WFDC2/HE4 in serum, plasma, and cell culture supernatant.

Individual users should determine appropriate conditions when using other types of samples.

This assay kit is for research use only and not for use in diagnostic or therapeutic procedures.

Storage

- Upon receipt store all components at 4°C.
- Do not expose reagents to excessive light.

Introduction

WFDC2 (WAP Four-disulfide core domain protein 2), also known as HE4 (Human Epididymis protein 4) is a member of the whey acidic protein (WAP) family of molecules. Mature human WFDC2/HE4 is a small secretory glycoprotein which is detectable in human serum. This protein contains two WAP domains that likely mediate anti-protease and/or anti-microbial activity. WFDC2/HE4 is expressed by a wide variety of epithelial cells, including male reproductive system, regions of the respiratory tract and nasopharynx (1). Correspondent to its high expression in cancer cells arising from ovarian (3), breast (4), and lung tissues (2,5), serum levels of WFDC2/HE4 are elevated in these cancer patients. These observation made WFDC2/HE4 a potential diagnostic marker of tissue-specific cancer. Furthermore, it is reported that serum level of WFDC2/HE4 is related to heart failure severity (6). Though WFDC2/HE4 is considered to play a role in cell proliferation and cancer cell malignancy (7), its mechanism is remain unclear.

Principle of the Assay

The MBL Research Product **CircuLex Human WFDC2/HE4 ELISA Kit** employs the quantitative sandwich enzyme immunoassay technique. An antibody specific for human WFDC2/HE4 is pre-coated onto a microplate. Standards and samples are pipetted into the wells and the immobilized antibody binds any human WFDC2/HE4 present. After washing away any unbound substances, an HRP conjugated monoclonal antibody specific for human WFDC2/HE4 is added to the wells. Following a wash to remove any unbound antibody HRP conjugate, the remaining conjugate is allowed to react with the substrate H_2O_2-tetramethylbenzidine. The reaction is stopped by addition of acidic solution and absorbance of the resulting yellow product is measured at 450 nm. The absorbance is proportional to the concentration of human WFDC2/HE4. A standard curve is constructed by plotting absorbance values versus human WFDC2/HE4 concentrations of calibrators, and concentrations of unknown samples are determined using this standard curve.

Summary of Procedure

Add 100 µL of diluted sample to the wells
↓ Incubate for 60 minutes at room temp.
Wash the wells
↓
Add 100 µL of HRP conjugated anti-human WFDC2/HE4 antibody
↓ Incubate for 60 minutes at room temp.
Wash the wells
↓
Add 100 µL of Substrate Reagent
↓ Incubate for 10-20 minutes at room temp.
Add 100 µL of Stop Solution
↓
Measure absorbance at 450 nm

Materials Provided

All samples and standards should be assayed in duplicate. The following components are supplied and are sufficient for the one 96-well microplate kit.

Microplate: One microplate supplied ready to use, with 96 wells (12 strips of 8-wells) in a foil, zip-lock bag with a desiccant pack. Wells are pre-coated with anti-human WFDC2/HE4 monoclonal antibody as a capture antibody.

10X Wash Buffer: One bottle containing 100 mL of 10X buffer containing Tween®-20

Dilution Buffer: One bottle containing 50 mL of 1X buffer; use for sample dilution. Ready to use.

Human WFDC2/HE4 Standard: One vial containing 1.5 ng of lyophilized recombinant human WFDC2/HE4

HRP conjugated Detection Antibody: One vial containing 12 mL of HRP (horseradish peroxidase) conjugated anti-human WFDC2/HE4 monoclonal antibody. Ready to use.

Substrate Reagent: One bottle containing 20 mL of the chromogenic substrate, tetra-methylbenzidine (TMB). Ready to use.

Stop Solution: One bottle containing 20 mL of 1 N H_2SO_4. Ready to use.

Materials Required but not Provided

- **Pipettors:** 2-20 µL, 20-200 µL and 200-1,000 µL precision pipettors with disposable tips.
- **Precision repeating pipettor**
- **Orbital microplate shaker**
- **Microcentrifuge and tubes** for sample preparation.
- **Vortex mixer**
- **(Optional) Microplate washer:** Manual washing is possible but not preferable.
- **Plate reader** capable of measuring absorbance in 96-well plates at dual wavelengths of 450 nm/540 nm. Dual wavelengths of 450/550 or 450/595 nm can also be used. The plate can also be read at a single wavelength of 450 nm, which will give a somewhat higher reading.
- **(Optional) Software package facilitating data generation and analysis**
- **500 or 1,000 mL graduated cylinder**.
- **Reagent reservoirs**
- **Deionized water of the highest quality**
- **Disposable paper towels**

Precautions and Recommendations

- **Although we suggest to conduct experiments as outlined below, the optimal experimental conditions will vary depending on the parameters being investigated, and must be determined by the individual user.**
- Allow all the components to come to room temperature before use.
- All microplate strips that are not immediately required should be returned to the zip-lock pouch, which must be carefully resealed to avoid moisture absorption.
- Do not use kit components beyond the indicated kit expiration date.
- Use only the microtiter wells provided with the kit.
- Rinse all detergent residue from glassware.
- Use deionized water of the highest quality.
- Do not mix reagents from different kits.
- The buffers and reagents in this kit may contain preservatives or other chemicals. Care should be taken to avoid direct contact with these reagents.
- Do not mouth pipette or ingest any of the reagents.
- Do not smoke, eat, or drink when performing the assay or in areas where samples or reagents are handled.
- Dispose of tetra-methylbenzidine (TMB) containing solutions in compliance with local regulations.
- Avoid contact with Substrate Solution which contains hydrogen peroxide.
- **CAUTION: Biological samples may be contaminated with infectious agents. Do not ingest, expose to open wounds or breathe aerosols. Wear protective gloves and dispose of biological samples properly.**
- **CAUTION: Stop Solution is a strong acid. Wear disposable gloves and eye protection when handling the solution.**

Sample Collection and Storage

Serum: Use a serum separator tube and allow samples to clot for 60 ± 30 minutes. Centrifuge the samples at 4°C for 10 minutes at 1,000 x *g*. Remove serum and assay immediately or store samples on ice for up to 6 hours before assaying. Aliquots of serum may also be stored below -70°C for extended periods of time. Avoid repeated freeze-thaw cycles.

Plasma: Collect plasma using EDTA-2Na or heparin as the anticoagulant. If possible, collect the plasma into a mixture of EDTA-2Na and Futhan5 to stabilize the sample against spontaneous *in vitro* complement activation. Immediately centrifuge samples at 4°C for 15 minutes at 1,000 x *g*. Assay immediately or store samples on ice for up to 6 hours before assaying. Aliquots of plasma may also be stored below -70°C for extended periods of time. Avoid repeated freeze-thaw cycles.

Note: Heparin and Citrate plasma has not been validated for use in this assay.

Cell culture supernatant: Remove any particulates by centrifugation and assay immediately or aliquot and store samples below -70°C. Avoid repeated freeze-thaw cycles.

Detailed Protocol

The MBL Research Product **CircuLex Human WFDC2/HE4 ELISA Kit** is provided with removable strips of wells so the assay can be carried out on separate occasions using only the number of strips required for the particular determination. Since experimental conditions may vary, an aliquot of the Human WFDC2/HE4 Standard within the kit, should be included in each assay as a calibrator. Disposable pipette tips and reagent troughs should be used for all liquid transfers to avoid cross-contamination of reagents or samples.

Preparation of Working Solutions

All reagents need to be brought to room temperature prior to the assay. Assay reagents are supplied ready-to-use, with the exception of **10X Wash Buffer** and **Human WFDC2/HE4 Standard**.

1. Prepare a working solution of **Wash Buffer** by adding **100 mL** of the **10X Wash Buffer** to **900 mL** of deionized (distilled) water (**ddH_2O**). Mix well. Store at 4°C for two weeks or -20°C for long-term storage.

2. Reconstitute **Human WFDC2/HE4 Standard** with **0.5 mL** of **ddH_2O**. by gently mixing. After reconstitution, immediately dispense it in small aliquots (e.g. 100 µL) to plastic micro-centrifuge tubes and store below -70°C to avoid non-specific adsorption to glass surface and multiple freeze-thaw cycles. The concentration of the reconstituted Human WFDC2/HE4 Standard should be **3.2 ng/mL**, which is referred to as a **Master Standard** of human WFDC2/HE4.

   Prepare Standard Solutions as follows:
   Use the **Master Standard** to produce a dilution series (below). Mix each tube thoroughly before the next transfer. **Std.1 (320 pg/mL)** serves as the highest standard. The **Dilution Buffer** serves as the zero standard (Blank).

	Volume of Standard	Dilution Buffer	Concentration
Std.1	60 µL of Master Standard (3.2 ng/mL)	540 µL	320 pg/mL
Std.2	300 µL of Std. 1 (320 pg/mL)	300 µL	160 pg/mL
Std.3	300 µL of Std. 2 (160 pg/mL)	300 µL	80 pg/mL
Std.4	300 µL of Std. 3 (80 pg/mL)	300 µL	40 pg/mL
Std.5	300 µL of Std. 4 (40 pg/mL)	300 µL	20 pg/mL
Std.6	300 µL of Std. 5 (20 pg/mL)	300 µL	10 pg/mL
Std.7	300 µL of Std. 6 (10 pg/mL)	300 µL	5 pg/mL
Blank	-	300 µL	0 pg/mL

Note: Do not use a Repeating pipette. Change tips for every dilution. Wet tip with Dilution Buffer before dispensing. Discard any unused Standard Solutions after use.

Sample Preparation

Dilute samples with **Dilution Buffer**.
- Serum and plasma samples may require a 50-fold dilution.
- Cell culture supernatants require neat to appropriate dilution.

Assay Procedure

1. Remove the appropriate number of microtiter wells from the foil pouch and place them into the well holder. Return any unused wells to the foil pouch, refold, seal with tape and store at 4°C.

2. Dilute samples with **Dilution Buffer**. (See "Sample Preparation" above.)

3. Pipette **100 µL** of **Standard Solutions (Std1-Std7, Blank)** and **diluted samples** in duplicates, into the appropriate wells.

4. Incubate the plate **at room temperature (ca.25°C) for 60 minutes**, shaking at ca. 300 rpm on an orbital microplate shaker.

5. Wash 4-times by filling each well with Wash Buffer (350 µL) using a squirt bottle, multi-channel pipette, manifold dispenser or microplate washer.

6. Add **100 µL** of **HRP conjugated Detection Antibody** into each well.

7. Incubate the plate **at room temperature (ca.25°C) for 60 minutes**, shaking at ca. 300 rpm on an orbital microplate shaker.

8. Wash 4-times by filling each well with Wash Buffer (350 µL) using a squirt bottle, multi-channel pipette, manifold dispenser or microplate washer.

9. Add **100 µL** of **Substrate Reagent**. Avoid exposing the microtiter plate to direct sunlight. Covering the plate with e.g. aluminum foil is recommended. Return Substrate Reagent to 4°C immediately after the necessary volume is removed

10. Incubate the plate **at room temperature (ca. 25°C) for 10-20 minutes**, shaking at ca. 300 rpm on an orbital microplate shaker. The incubation time may be extended up to 30 minutes if the reaction temperature is below than 20°C.

11. Add **100 µL** of **Stop Solution** to each well in the same order as the previously added Substrate Reagent.

12. Measure absorbance in each well using a spectrophotometric microplate reader at dual wavelengths of 450/540 nm. Dual wavelengths of 450/550 or 450/595 nm can also be used. Read the microplate at 450 nm if only a single wavelength can be used. Wells must be read within 30 minutes of adding the Stop Solution.

Note-1: Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.

Note-2: Reliable standard curves are obtained when either O.D. values do not exceed 0.2 units for the blank (zero concentration), or 2.5 units for the highest standard concentration.

Note-3: If the microplate reader is not capable of reading absorbance greater than the absorbance of the highest standard, perform a second reading at 405 nm. A new standard curve, constructed using the values measured at 405 nm, is used to determine the concentration of off-scale samples. The readings at 405 nm should not replace the on-scale readings at 450 nm.

Calculations

Average the duplicate readings for each standard, control and sample, and subtract the optical density of the average zero standard. Plot the optical density versus the concentration of standards and draw the best curve. Most microtiter plate readers perform automatic calculations of analyte concentration. The standard curve fits best to a sigmoidal four-parameter logistic equation. The results of unknown samples can be calculated with any computer program having a four-parameter logistic function.

A standard curve is also to be constructed by plotting the absorbance (Y) versus log of the known concentration (X) of standards, using a cubic function. Alternatively, the logit log function can be used to linearize the standard curve (i.e. logit of optical density (Y) is plotted versus log of the known concentration (X) of standards). To determine the concentration of each sample, first find the optical density on the y-axis and extend a horizontal line to the standard curve. At the point of intersection, extend a vertical line to the x-axis and read the corresponding concentration.

If the samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

Measurement Range

The measurement range is 5 pg/mL to 320 pg/mL. Any sample reading higher than the highest standard should be diluted with Dilution Buffer in higher dilution and re-assayed. Dilution factors need to be taken into consideration in calculating the human WFDC2/HE4 concentration.

Troubleshooting

1. All samples and controls should be assayed in duplicate, using the protocol described in the **Detailed Protocol**. Incubation times or temperatures significantly different from those specified may give erroneous results.

2. Poor duplicates, accompanied by elevated values for wells containing no sample, indicate insufficient washing. If all instructions in the **Detailed Protocol** were followed accurately, such results indicate a need for washer maintenance.

3. Overall low signal may indicate that desiccation of the plate has occurred between the final wash and addition of Substrate Reagent. Do not allow the plate to dry out. Add Substrate Reagent immediately after wash.

Reagent Stability

All of the reagents included in the MBL Research Product **CircuLex Human WFDC2/HE4 ELISA Kit** have been tested for stability. Reagents should not be used beyond the stated expiration date.

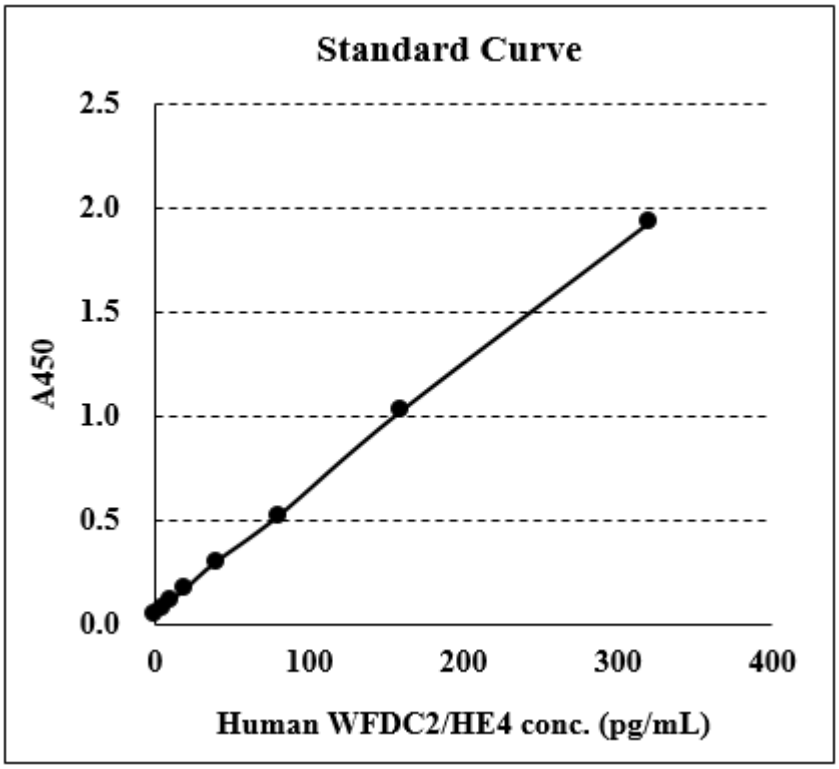
Assay Characteristics

1. Sensitivity

The limit of detection (defined as such a concentration of human WFDC2/HE4 giving absorbance higher than mean absorbance of blank* plus three standard deviations of the absorbance of blank: A blank + 3SD blank) is better than 3.7 pg/mL of sample.

* Dilution Buffer was pipetted into blank wells.

Typical standard curve



2. Precision

Intra-assay Precision (Precision within an assay)

Three samples* of known concentration were tested sixteen times on one plate to assess intra-assay precision.

• Intra-assay (Within-Run, n=16) CV=3.0-3.9%

* Sample: Cell culture supernatant

	Human WFDC2/HE4 conc. (pg/mL)		
	Sample 1	Sample 2	Sample 3
1	245.7	158.1	74.3
2	235.2	170.1	70.0
3	232.8	157.1	67.3
4	240.9	163.1	69.2
5	236.9	164.5	65.9
6	244.5	167.2	68.8
7	246.5	169.6	73.1
8	251.5	161.4	68.9
9	236.6	176.4	68.0
10	246.5	177.0	70.2
11	237.2	172.4	70.8
12	234.5	171.6	69.7
13	253.7	175.5	74.4
14	239.8	172.9	69.4
15	256.7	172.2	70.8
16	240.7	162.1	76.0
MAX.	**256.7**	**177.0**	**76.0**
MIN.	**232.8**	**157.1**	**65.9**
MEAN	**242.5**	**168.2**	**70.4**
S.D.	**7.2**	**6.4**	**2.8**
C.V.	**3.0%**	**3.8%**	**3.9%**

Inter-assay Precision (Precision between assays)

Three samples* of known concentration were tested in five separate assays to assess inter-assay precision.

• Inter-assay (Run-to-Run, n=5) CV=7.6-9.9 %

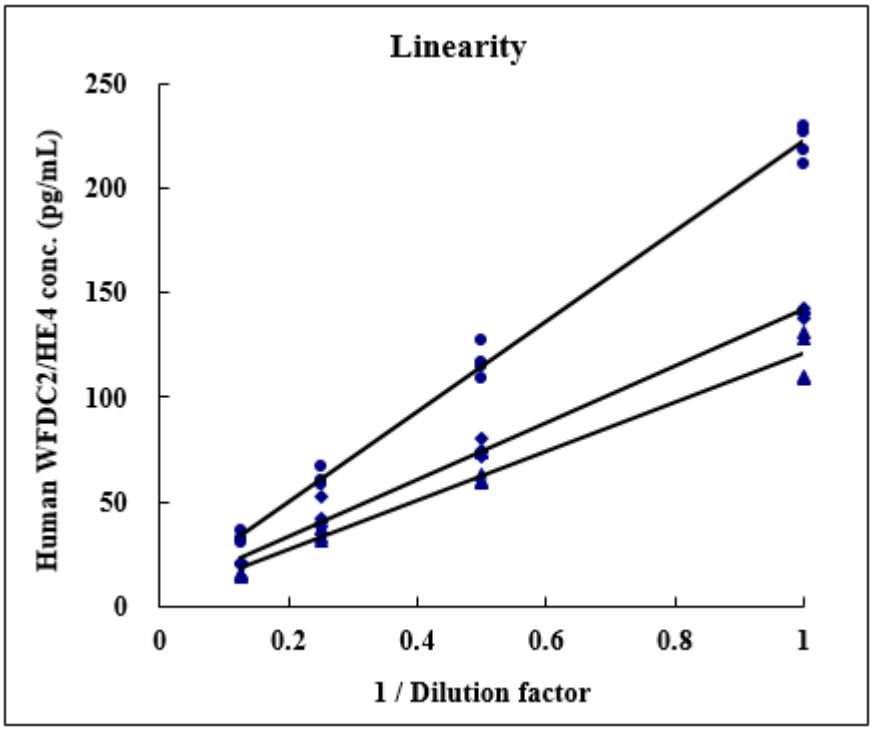
* Sample: Cell culture supernatant

	Human WFDC2/HE4 conc. (pg/mL)		
	Sample 1	Sample 2	Sample 3
1	71.1	160.5	227.4
2	63.5	143.2	221.1
3	71.3	148.3	215.0
4	73.7	121.4	241.3
5	62.0	146.2	191.5
MAX.	**73.7**	**160.5**	**241.3**
MIN.	**62.0**	**121.4**	**191.5**
MEAN	**68.3**	**143.9**	**219.3**
S.D.	**5.2**	**14.2**	**18.3**
C.V.	**7.6%**	**9.9%**	**8.4%**

3. Linearity

To assess the linearity of the assay, three samples* were serially diluted with Sample/Standard Dilution Buffer to produce samples with values within the dynamic range of the assay.

*Sample: Cell culture supernatant



Example of Test Results

Fig.1 Human WFDC2/HE4 concentration in sera from healthy volunteers.

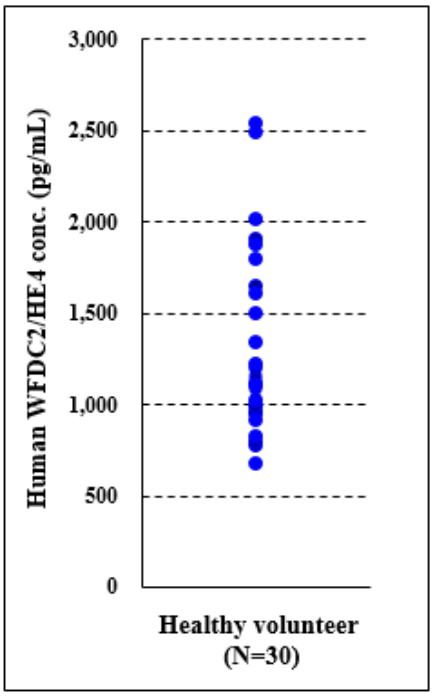
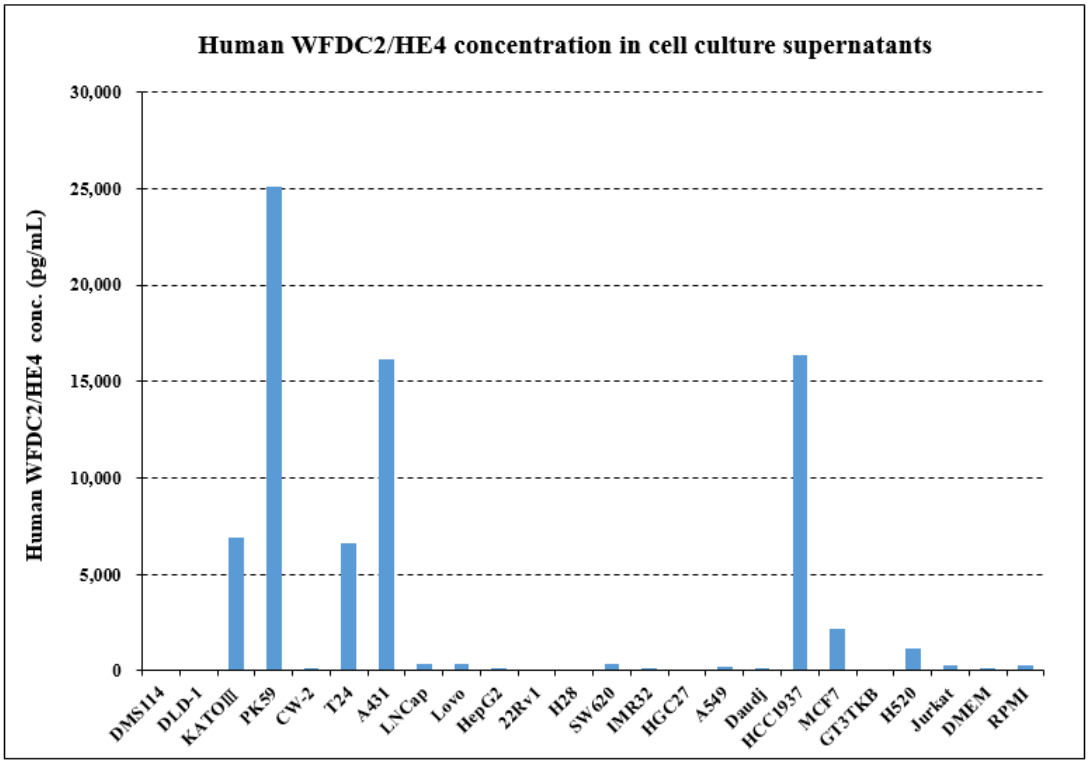


Fig.2 Human WFDC2/HE4 concentration in cell culture supernatants



References

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3. Hellström I *et al*. The HE4 (WFDC2) protein is a biomarker for ovarian carcinoma. Cancer Res. 2003 Jul 1;63(13):3695-700.

4. Kamei M *et al*. HE4 expression can be associated with lymph node metastases and disease-free survival in breast cancer. Anticancer Res. 2010 Nov;30(11):4779-83.

Related Products

*** CircuLex Human FAM3D ELISA Kit: Cat# CY-8201**
*** CircuLex Human PAP1/REG3α ELISA Kit: Cat# CY-8203**
*** CircuLex Human MICA ELISA Kit: Cat# CY-8206**
*** CircuLex Human MICB ELISA Kit: Cat# CY-8207**
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*** CircuLex Human REG4 ELISA Kit: Cat# CY-8213**
*** CircuLex Human Elafin/SKALP ELISA Kit: Cat# CY-8214**
*** CircuLex Human soluble Nectin-4 ELISA Kit: Cat# CY-8217**
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*** CircuLex Human Amphiregulin/AREG ELISA Kit: Cat# CY-8220**
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