



## The fabrication of magnetic particle-based chemiluminescence immunoassay for human epididymis protein-4 detection in ovarian cancer



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### ABSTRACT

The magnetic particles have a significant influence on the immunoassay detection and cancer therapy. Herein, the chemiluminescence immunoassay combined with the magnetic particles (MPCLIA) was presented for the clinical determination and analysis of human epididymis protein 4 (HE4) in the human serum. Under the optimized experiment conditions, the secure MPCLIA method can detect HE4 in the broader range of 0–1000 pmol/L, with a lower detection limit of 1.35 pmol/L. The satisfactory recovery rate of the method in the serum ranged from 83.62% to 105.10%, which was well within the requirement of clinical analysis. Moreover, the results showed the good correlation with enzyme-linked immunosorbent assay (ELISA), with the correlation coefficient of 0.9589. This proposed method has been successfully applied to the clinical determination of HE4 in the human serum.

### 1. Introduction

Epithelial ovarian cancer, which is one of the most commonly diagnosed gynecologic malignancy and the highest mortality rate, threatens the human health and life quality [1–6]. Therefore, timely screening and detecting ovarian cancer in the earlier stage could be the significant approach to reduce the mortality. However, lack of clinical symptoms and due to the low incidence in early stage hinder the occurrence of detection sensitivity [7,8]. Since the early and accurate prognosis analysis is the fundamental premise to improve the survival rates of patients with ovarian cancer, therefore it is highly demanded biomarkers with higher diagnostic accuracy, and setting up the sensitive and reliable analytical methods to monitor ovarian cancer in a timely and accurate way in patients.

Recently, human epididymis protein-4, also named as whey-acidic-protein four-disulfide core protein-2 (WFDC2), is one of the most promising new biomarkers [9,10] and has been approved by the Food and Drug Administration (FDA) as the sensitive serum biomarkers for the early diagnosis and monitoring of epithelial ovarian cancer. To date, various analysis methods have been widely established for the serum

HE4 detection, including enzyme-linked immunosorbent assay (ELISA), electrochemiluminescence enzyme immunoassay, time-resolved fluoroimmunoassay (TRFIA), amplified luminescent proximity homogeneous immunoassay (AlphaLISA) [11–14]. Despite the considerable advancements in technology, many disadvantages still exist. ELISA and TRFIA are inferior regarding of sensitivity and accuracy. Hence, a more sensitive and convenient screening method is required in the clinical diagnoses.

Currently, chemiluminescence immunoassay (CLIA) is recognized as the most sensitive classical method in immunologic diagnosis [15,16], with the significant merits of high sensitivity, low noise, broader linearity, reduced assay time, free of radioactive reagents, and easy to use. Meanwhile, the light intensity of chemiluminescence (CL) reaches its maximum within 1–2 min after substrate addition, thus shortening the overall analytical procedure when compared with the conventional colorimetric assays. CLIA has also been widely used in the clinical detection of tumor markers, such as alpha-fetoprotein (AFP), prostate-specific antigen (PSA), carbohydrate antigen 125 (CA125), and neuron-specific enolase (NSE) [17–20]. At the same time, to further improve the detection efficiency and detection time, magnetic particles were

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introduced. Magnetic particles, which equipped with micron- or nano-scale iron oxide as the core component, magnetic particles can rapidly aggregate under an external magnetic field. When the external magnetic field is removed, the magnetic particles will be re-suspended in solution, which dramatically reduces the cleaning time and easy to automate. On the other hand, MPs could possess remarkable advantages, containing the large surface area, magnetic susceptibility, low toxicity, low cost of synthesis, compatibility with biomaterials and easy to separate from the matrix.

In this work, HE4-antibody(Ab)-alkaline phosphatase (ALP) were prepared by utilizing an improved labeling method with higher efficiency. By combining the monoclonal antibody-coated magnetic beads, a specific and sensitive determination for HE4 was developed by chemiluminescence immunoassays. The specificity and repeatability of the immunoassay were investigated by the cross-reactivity and the recycle experiments. The results obtained were in an excellent linear relationship with those from commercial ELSA kits.

## 2. Materials and methods

### 2.1. Chemicals, buffers, calibrators, and samples

1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide hydrochloride (EDC), N-Hydroxysuccinimide (NHS), bovine serum albumin (BSA), Dimethyl sulfoxide(DMSO), O-(carboxymethyl) hydroxylamine hemihydrochloride (CMO), N, N-dimethylformamide (DMF), 2-Aminoethanol were provided from Sigma-Aldrich, Sephadex G-25 was bought from GE Health Life Sciences. Alkaline phosphatase (ALP) was procured from BBI Enzymes. 4-(N-Maleimidomethyl) cyclohexanecarboxylic acid N-hydroxysuccinimide ester was purchased from Thermo. Lumigen APS-5 was purchased by Lumigen, Inc. tris (hydroxymethyl) aminomethane hydrochloride was from Sinopharm Chemical Reagent Co., Ltd. All chemicals were used without further treatment.

The phosphate buffer (pH 7.4) contained the 0.01 M  $\text{KH}_2\text{PO}_4$  and 0.01 M  $\text{Na}_2\text{HPO}_4$ ; the washing buffer (PBST): PBS buffer with 0.5 mL/L Tween 20.

The commercial HE4 ELISA assay kit was bought from Cusabio Biotech. Co., LTD. CEA, AFP, carbohydrate antigen 125 (CA125), carbohydrate antigen 153 (CA153), carbohydrate antigen199 (CA199) were bought from HyTest biotech Co. LTD. Elafin, Secretory leukocyte protease inhibitor(SLPI) were purchased from US Biological Life Sciences (America), Bilirubin, Hemoglobins, Triglycerides were bought from Solarbio Science &Technology Co., LTD. Rheumatoid factor was provided from Beijing Labo Biotech. Co., LTD.

### 2.2. Instrumentation

The immunomagnetic particles were provided by Merck (Beijing, China), which the diameter was 1.0  $\mu\text{m}$ . The magnetic separator was provided by the Tianjin Baseline Chromtech Research Centre (Tianjin, China). The chemiluminescence analyzer (BHP9507, Beijing Hamamatsu Photon Techniques Inc, China) were utilized to estimate the chemiluminescence (CL) signal.

### 2.3. Methods

#### 2.3.1. Preparation of monoclonal antibody-coated magnetic beads

The conjugates of the magnetic beads and antibody were prepared as follows. To activate carboxyl groups on the beads, 230  $\mu\text{L}$  fresh EDC (10 mg/mL) and 260  $\mu\text{L}$  NHS (10 mg/mL) were added to 2 mg magnetic beads in 1 mL coating buffer, reacting for 30 min at room temperature. The activation solution was then discarded, and the magnetic beads were washed with the solution for several times. Activated magnetic beads have the ability to couple with biological ligands via primary amines.

Afterward, 100  $\mu\text{g}$  monoclonal antibody was added in 1 mL binding buffer and both were mixed with the above activated magnetic beads by gentle rotation (60 rpm) overnight at room temperature. Then the supernatant was removed by the magnetic separator and the beads suspended was blocked. The washing process was repeated three times followed by incubation with 1 mL blocking buffer for 3 h at room temperature. After the final washing step, the antibody-magnetic bead conjugates were resuspended in 5% MCHE-020 buffer and stored at 4  $^\circ\text{C}$  until required.

#### 2.3.2. Preparation of HE4-Antibody(Ab)-Alkaline phosphatase (ALP)

Before coupling with ALP, HE4-antibody was activated. Briefly, 1 mg antibody in 0.05 M PBS (pH 8.0, 5 mg/mL) was incubated with the 2IT solution at room temperature for 30 min and purified by a Sephadex G-25 column primed by 0.05 M PBS (pH 7.3).

2 mg ALP was activated by 10  $\mu\text{L}$  of 10 mg/mL SMCC for 30 min at room temperature. The activated detection antibody was mixed with the pretreated ALP. Excess functional groups in SMCC were blocked by maleimide (50  $\mu\text{L}$ , 10 mg/mL). The reaction of antibody and ALP was performed in the dark for 12 h at room temperature. The labeled antibody mixture was dialyzed in PBS (pH 6.5) overnight and purified by Sephadex G-25. Finally, the purified labeled antibody was stored at 4  $^\circ\text{C}$ , denoted as HE4-Ab-ALP.

#### 2.3.3. Immunoassay procedure

The schematic diagram of the MPCLIA analysis was shown in Fig. 1, first of all, 300  $\mu\text{L}$  of standard solutions or samples, 60  $\mu\text{L}$  monoclonal

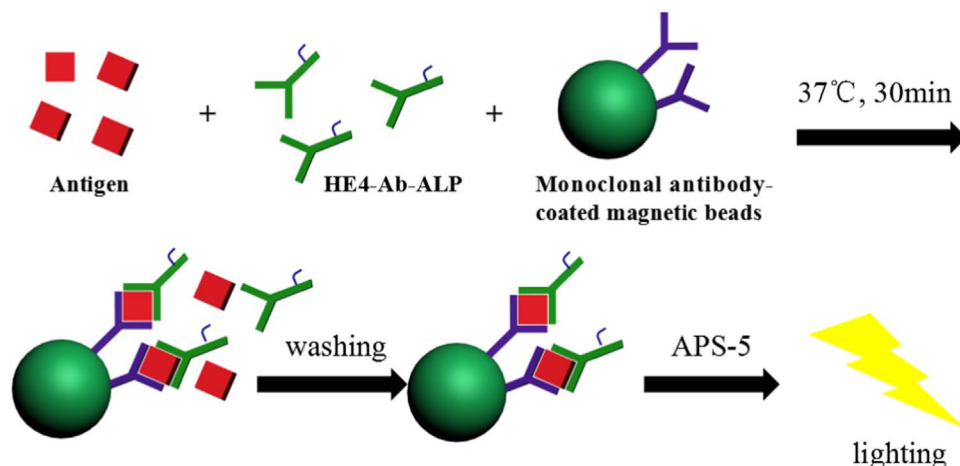


Fig. 1. The schematic principle of the magnetic particle based-chemiluminescence immunoassay.

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