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Capillary Electrophoretic Immunoassay with Laser-induced Fluorescence Detection for Interferon-gamma

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Abstract: Capillary electrophoretic immunoassay with laser-induced fluorescence detection for recombinant human interferon-gamma (IFN-γ) was established. The limits of detection for three forms of IFN-γ are 6.9 ng/L, 5.7 ng/L and 5.0 ng/L, respectively.

Keywords: Capillary electrophoretic immunoassay, interferon-gamma.

Interferon-gamma (IFN-γ) is a multifunctional protein with three forms, *e.g.* IFN-γ glycosylated at a single site or both sites and non glycosylated. All these kind of IFN-γ take an important role in immunity adjustment[1]. Comparing with conventional immunoassays, capillary electrophoretic immunoassay (CEIA) with laser-induced fluorescence (LIF) detection possesses several advantages such as high sensitivity, less sample consumption, high separation efficiency and facility to automation[2]. The determination of IFN-γ by CEIA-LIF detection has not been reported yet.

In the present work, a non-competitive format was used. An excess amount of anti-IFN-γ monoclonal antibody labeled with fluorescein isothiocyanate (FITC) (Ab*) in phosphate-buffered saline (PBS) was added to the solution containing recombinant human IFN-γ (Ag) in PBS. After the completion of the immuno-reaction, the complexes of the three forms of IFN-γ with Ab* were formed. The complexes (Ab*-Ag) and free Ab* were separated by capillary electrophoresis (CE). The separation buffer consisted of 1.0×10^{-2} mol/L borax-9.3×10^{-2} mol/L boric acid-8.0×10^{-5} mol/L spermine (pH 8.0). CEIA-LIF was performed using a CE system with a LIF detector (488 nm Laser Module) (P/ACE MDQ, Beckman Coulter, Fullerton CA, USA).

The electropherograms of the solution containing Ab* and Ab*-Ag are shown in **Figure 1**. With increasing IFN-γ, the areas of peaks 1, 2 and 3 increased and the area of peak 4 decreased. Therefore, peak 1, 2 and 3 corresponded to the complexes of three forms of Ag with Ab*, respectively, and peak 4 is the peak of Ab*. Since the standard was a mixture of the three forms of IFN-γ, we could not identify them

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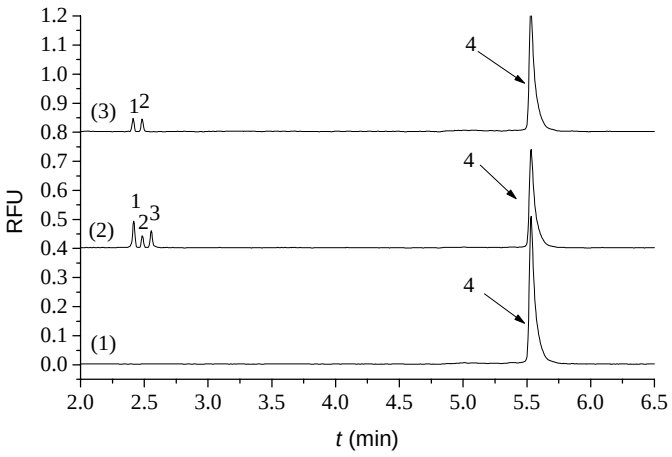
Figure 1 Electropherograms of the solution containing Ab* and Ab*-Ag.



(1) 1×10^{-4} g/L Ab*, (2) (1)+1×10^{-6} g/L Ag, (3) (1)+Sample. Incubation for 15 min at room
(2) temperature. Capillary, 31.2 cm×50 μm I.D., Effective length, 21 cm; Coolant tubing, 14 cm; Separation voltage, 20 kV; Coolant, 20℃, Sample temperature, 10℃, Injection, 0.5 psi×3 s.

accurately. By comparison with the electropherogram (curve 3) of the extract of the NK cell with only two glycosylated forms of IFN-γ, the peak 3 in curve 2 should be the non glycosylated IFN-γ. Based on the area ratios of these three peaks, the amount ratios of the three forms could be calculated to be 52%, 19% and 29%, respectively. The linear range for the three forms of IFN-γ were 6.9×10^{-9}~2.1×10^{-6} g/L, 5.7×10^{-9}~7.6×10^{-7} g/L and 5.0×10^{-9}~1.2×10^{-6} g/L, respectively. The detection limits were 6.9×10^{-9}, 5.7×10^{-9} and 5.0×10^{-9} g/L, respectively. The relative standard deviations were 1.4~1.6% for peak area. The two forms of IFN-γ in the extract of NK cell were quantified as 1.0 μg/L and 0.95 μg/L by the standard addition method. The total IFN-γ concentration was 1.95 μg/L, which agreed with the value (1.98 μg/L) detected by ELISA.

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