Development of Fluorescence-linked Immunosorbent Assay for High Throughput Screening of Interferon-γ

http://www.firstlight.cn 2006-03-16

Background: Human interferon-gamma (hIFN-γ) is produced by lymphocytes and has a variety of biological properties. Measurement of hIFN-γ is widely used for various immunological responses for allergic or autoimmune diseases. Enzyme-linked immunosorbent assay (ELISA) is an established immunoassay used to quantify cellular metabolites or cytokines. ELISA requires many incubation and wash steps and is not practically suitable for screening large numbers of samples.

Methods: We have developed a fluorescence-linked immunosorbent assay (FLISA) method for the detection of hIFN-γ. We measured the 50% inhibitory concentration (IC50) value of the hIFN-γ production by interleukin (IL)-18 binding protein and anti-IL-18 monoclonal antibody. The IC50 described by FLISA was compared with that by ELISA.

Results: We developed a new system for measuring hIFN-γ using Allophycocyanine (APC) fluorescent protein and compared it with the previous method using Cy5.5. The proposed FLISA had a smaller coefficient of variation than ELISA, and the means of coefficient of variation using the same samples measured by ELISA and FLISA were, respectively, 11.1% and 3.8%, suggesting that the edge effect often giving non-specific results may be smaller in FLISA than in ELISA.

Conclusions: The improved FLISA system proposed is ideally suited for efficient measurements of hIFN-γ. This homogeneous and multiplex method will be a powerful tool for high throughput screening for drug discovery research.

存档文本