



IL-4 (INTERLEUKIN 4)

The Singulex IL-4 assay is sensitive enough to quantify IL-4 concentrations in plasma from healthy, normal human subjects with a level of accuracy and precision currently unobtainable using other high sensitivity assays.

BIOLOGY AND DISEASES

Interleukin-4 (IL-4), is a cytokine that is a key regulator in humoral and adaptive immunity. IL-4 induces differentiation of naive helper T-cells (Th0 cells) to Th2 cells. It has many biological roles, including the stimulation of activated B-cell and T-cell proliferation, and the differentiation of CD4+ T-cells into Th2 cells. IL-4 plays an important role in the development of allergic inflammatory responses. IL-4 controls the production of IgE, expands IL-4 producing T-cell subsets, and stabilizes effector cell functions.

THERAPIES

IL-4 has therapeutic potential due to its role in the development of allergic inflammatory responses. IL-4 also has shown to have promise in drug targeting for cancer. For example, PRX321 (Protox) is a targeted therapeutic toxin in which IL-4 is linked to a *Pseudomonas* exo-toxin, a potent substance that can destroy cancer cells. Besides brain, kidney and lung cancer, PRX321 has shown promising preclinical results in a number of cancers over-expressing IL-4 receptors, including pancreatic, ovarian, breast, head and neck, melanoma, prostate and blood cancers such as chronic lymphocytic leukemia (CLL) and Hodgkin's lymphoma.

UNMET NEED

The concentration of plasma IL-4 in healthy human subjects has yet to be defined. Thus it is difficult to understand the role that differences in IL-4 concentrations play between disease and healthy states. In addition, when experimental therapeutics are used to target and decrease IL-4, measuring their efficacy by the velocity of IL-4 decreases is hindered by lack of assay sensitivity. Furthermore, the reading range of the most sensitive ELISA is limited to less than two logs, which forces sample retesting and wastage. Thus there is great need for a highly sensitive assay that can detect the velocity of subtle changes in concentration and measure baseline concentration of IL-4 in normal subjects.

SINGULEX ANSWER

Singulex's IL-4 assay enables the quantification of very low levels of plasma IL-4. The assay has an LLoQ of 0.28 pg/mL and a reading range of 0.12-2000 pg/mL, allowing measurement of small changes in IL-4 levels that can provide insights into therapeutic efficacy.

This assay will allow investigators to:

1. Measure the efficacy and dosing of therapeutics designed to interfere with general inflammatory and allergic responses.
2. Design more robust clinical and preclinical studies when IL-4 concentration is used as a therapeutic endpoint.
3. Understand how IL-4 levels change in patients as they transition from a healthy to diseased state.

ERENNA TECHNOLOGY ACCESS PROGRAM.

Through the Erenna Technology Access Program (ETAP), Singulex offers an interactive, results-driven solution to biomarker challenges faced by the pharmaceutical industry during product development. Singulex assists the development programs of our ETAP collaborators by developing customer-driven assays and access to a menu of fully-validated assays. Participants in ETAP gain access to the Singulex Erenna Immunoassay System, our proven expertise developing high-value immunoassays and our world-class customer support. Together with Singulex, our ETAP collaborators are expanding the utility of protein biomarkers and using them as tools to measure disease progression, drug efficacy and toxicity.

TABLE 1: Analytical sensitivity of the Singulex IL-4 assay.

Lower Limit of Detection (LoD)	0.12 pg/mL
Lower Limit of Quantification (LLoQ)	0.28 pg/mL
Reading Range	0.12–2000 pg/mL

Erenna® System



TABLE 2: IL-4 assay low-end standard curve data.

[IL-4] pg/mL	Detected Events	Std Dev	CV
2.19	1566	80	5%
1.09	911	28	3%
0.55	566	33	6%
0.28	386	29	7%
0.14	297	33	11%
0.07	256	14	6%
0.03	231	16	7%
0.00	208	35	17%

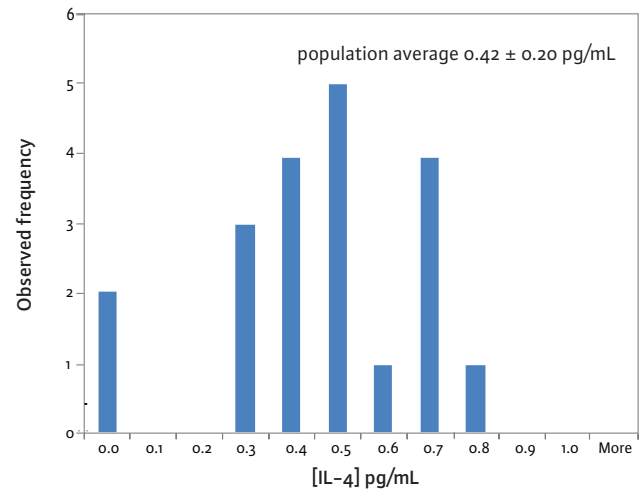


FIGURE 1: Plasma IL-4 concentration in healthy human subjects.

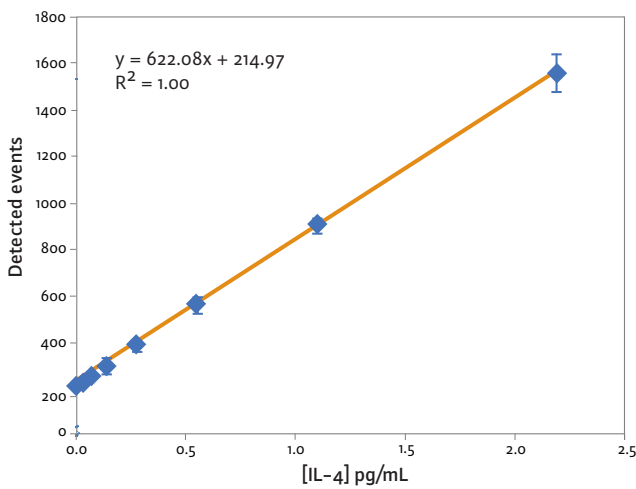


FIGURE 2: IL-4 low-end standard curve signal.

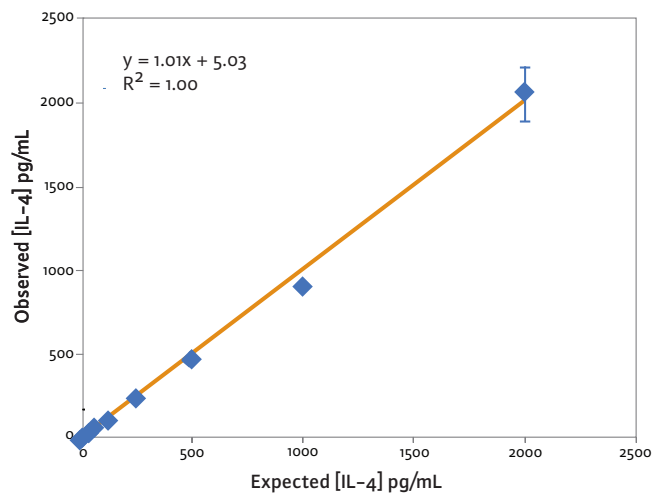


FIGURE 3: IL-4 assay curve fit.

These standard curves are for representational purposes only. A standard curve must be run with each assay.

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