

## mTNF $\alpha$ (TUMOR NECROSIS FACTOR-ALPHA, MOUSE)

The Singulex Mouse TNF $\alpha$  assay is sensitive enough to quantify mTNF $\alpha$  concentrations in small volume plasma samples from healthy mice, enabling better preclinical study design and long-term monitoring of individual mice over time.

### BIOLOGY AND DISEASES

Tumor necrosis factor alpha (TNF $\alpha$ ) is a pro-inflammatory cytokine with cytotoxic activity that is primarily produced by macrophages. TNF $\alpha$  is involved in systemic inflammation and is a member of the group of cytokines that stimulate the acute phase reaction. When administered to animals or humans it causes inflammation, fever, cardiovascular effects, hemorrhage, coagulation, and acute phase responses similar to those seen during acute infections and shock states. By promoting the inflammatory response, TNF $\alpha$  is associated with many autoimmune disorders such as rheumatoid arthritis, ankylosing spondylitis, Crohn's disease, psoriasis, and refractory asthma. TNF $\alpha$  also plays a role in cancer and regulates apoptotic cell death, cellular proliferation, differentiation, tumorigenesis, and viral replication.

### THERAPIES

Autoimmune disorders can be treated with TNF $\alpha$ -inhibitors, which act as anti-inflammatory agents. This inhibition can be achieved with a monoclonal antibody such as infliximab (Remicade) or adalimumab (Humira), or with a circulating receptor fusion protein such as etanercept (Enbrel). Modifications of the TNF $\alpha$  pathway may also have potential for treatment or prevention of some cancers; however, TNF $\alpha$ -inhibitors have also been implicated in the development of childhood leukemia.

### UNMET NEED

The broad involvement of inflammation in human disease ensures that TNF $\alpha$  will remain an attractive therapeutic target for auto-inflammatory diseases like rheumatoid arthritis and Crohn's disease, as well as other diseases like cancer, Alzheimer's, AIDS, tuberculosis, and insulin resistance. This presents a need to accurately quantify TNF $\alpha$  during preclinical and early phase drug development. In particular, there is a need for a more sensitive mouse TNF $\alpha$  assay that accurately quantifies mTNF $\alpha$  in very small volumes of mouse plasma to enable long-term, time-lapse monitoring of mice.

### SINGULEX ANSWER

The Singulex Mouse TNF $\alpha$  assay, optimized for use on the Erenna System, provides the sensitivity to quantify subpicogram levels of mTNF $\alpha$  in small volumes of plasma (<100  $\mu$ L). The precision of the mTNF $\alpha$  assay enables measurement of mTNF $\alpha$  velocity, providing valuable information on drug efficacy and disease progression.

The mTNF $\alpha$  assay has an LoD of 0.49 pg/mL and a reading range of 0.1 to 1000 pg/mL, providing accurate quantification of mTNF $\alpha$  in only 20  $\mu$ L of plasma.

This assay will allow investigators to:

1. Measure the efficacy and dosing of therapies that interfere with the inflammatory response, such as TNF $\alpha$  inhibitors.
2. Perform robust preclinical studies in mice when mTNF $\alpha$  concentration is used as a therapeutic endpoint.
3. Quantify mTNF $\alpha$  in very small volumes of mouse plasma to enable long-term, time-lapse monitoring of mice.

### 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 mTNF $\alpha$  assay.

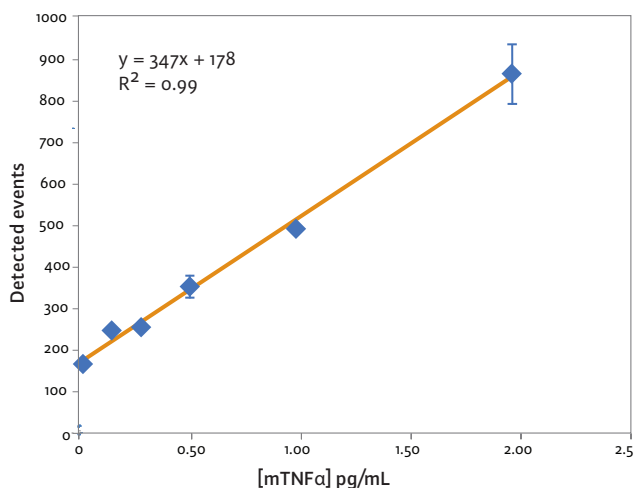
Lower Limit of Detection (LoD)	0.10 pg/mL
Lower Limit of Quantification (LLoQ)	0.49 pg/mL
Reading Range	0.10–1000 pg/mL

**Erenna<sup>®</sup> System**

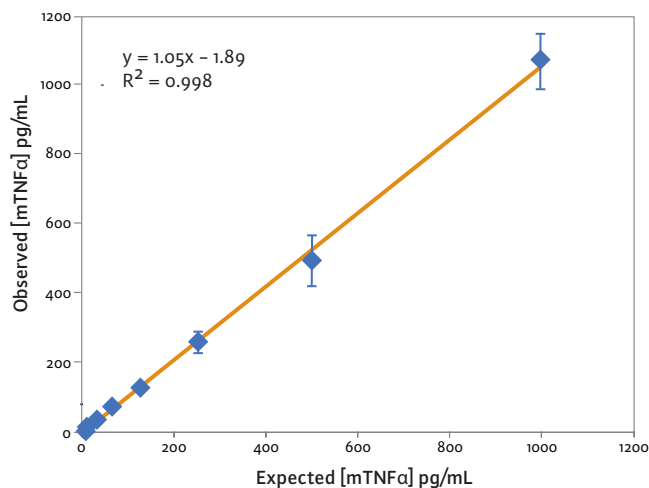


**TABLE 2:** mTNF $\alpha$  assay low-end standard curve data.

[mTNF $\alpha$ ] pg/mL	Detected Events	Std Dev	CV
1.95	864	71	8%
0.98	492	10	2%
0.49	358	28	8%
0.24	247	18	7%
0.12	254	16	6%
0.00	163	15	9%



**FIGURE 1:** mTNF $\alpha$  assay low-end standard curve signal.



**FIGURE 2:** mTNF $\alpha$  assay curve fit.

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

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