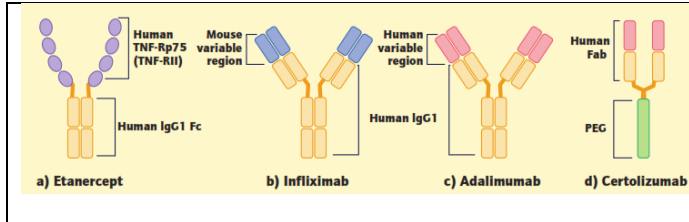


Humanized Anti-TNF-alpha (Humira/Adalimumab) Assay Kits

Tumor necrosis factor (TNF, or TNF- α) is a cytokine involved in systemic inflammation. TNF was thought to be produced primarily by macrophages, but it is produced also by a broad variety of cell types including lymphoid cells, mast cells, endothelial cells, cardiac myocytes, adipose tissue, fibroblasts, and neuronal tissue. Large amounts of TNF are released in response to lipopolysaccharide, other bacterial products, and Interleukin-1 (IL-1). In the skin, mast cells appear to be the predominant source of pre-formed TNF, which can be released upon inflammatory stimulus (e.g., LPS). It is a 212-amino acid-long type II transmembrane protein arranged in stable homotrimers. From this membrane-integrated form the soluble homotrimeric cytokine (sTNF) is released via proteolytic cleavage by the metalloprotease TNF alpha converting enzyme (TACE, also called ADAM17). TNF can bind two receptors, TNF-R1 (TNF receptor type 1; CD120a; p55/60) and TNF-R2 (TNF receptor type 2; CD120b; p75/80). TNF-R1 is expressed in most tissues, and can be fully activated by both the membrane-bound and soluble trimeric forms of TNF, whereas TNF-R2 is found only in cells of the immune system, and respond to the membrane-bound form of the TNF homotrimer.

The primary role of TNF is in the regulation of immune cells. TNF, being an endogenous pyrogen, is able to induce fever, to induce apoptotic cell death, to induce sepsis (through IL1 & IL6 production), to induce cachexia, induce inflammation, and to inhibit tumorigenesis and viral replication. TNF promotes inflammatory response, which in turn causes many of the clinical problems associated with autoimmune disorders such as rheumatoid arthritis, spondylitis, Crohn's disease, psoriasis, hidradenitis suppurativa and refractory asthma. These disorders are treated by using a **TNF inhibitor**. Inhibition can be achieved with a monoclonal antibody such as infliximab (Remicade), adalimumab (Humira) or certolizumab pegol (Cimzia).



Therapeutic TNF blockers: **Etanercept** (Enbrel) is 150 kda, p75 TNF-receptor domain-Fc (IgG1) fusion protein. **Infliximab** (Remicade) is mouse Fab-human Fc chimeric antibody (~150 kda). **Adalimumab** ~148 kda fully humanized antibody. **Certolizumab** pegol (Cimzia) has human mab (Fab) linked to PEG. **Golimumab** (CANTO 148/Simponi) is human mab. **CEP-37247** is the first human framework domain antibody (VL dAb 11-13 kda domains fused with human FC, total mol wt ~78 kda).



Adalimumab was the first fully human monoclonal antibody drug approved by the FDA. It was derived from phage display. Humira is a recombinant human IgG1k monoclonal antibody with human derived heavy and light chain variable regions and human IgG1:k constant regions. It consists of 1330 amino acids (~148 Kda). Humira binds to a single epitope on the N-terminus of the TNF-alpha and blocks its interaction with the p55 and p75 cell surface TNF-receptors. Adalimumab also lyses surface TNF expressing cells in vitro in the presence of complement. Adalimumab does not bind or inactivate lymphotoxin (TNF-beta). Treatment with HUMIRA may result in the formation of autoantibodies and, rarely, in the development of a lupus-like syndrome. In the rheumatoid arthritis controlled trials, 12% of patients treated with HUMIRA and 7% of placebo-treated patients that had negative baseline ANA titers developed positive titers at week 24. Approximately 5% (58 of 1062) of adult rheumatoid arthritis patients receiving HUMIRA developed low-titer antibodies to adalimumab at least once during treatment, which were neutralizing in vitro.

Adalimumab/Humira was constructed from a fully human monoclonal antibody, while infliximab is a mouse-human chimeric antibody and etanercept is a TNF receptor-IgG fusion protein. Adalimumab has been approved by the FDA for the treatment of rheumatoid arthritis, psoriatic arthritis, ankylosing spondylitis, Crohn's disease, moderate to severe chronic psoriasis and juvenile idiopathic arthritis.

Because adalimumab/humira suppresses TNF, which is part of the immune system, latent infections, such as tuberculosis, can be reactivated, and the immune system may be unable to fight new infections. This has led to fatal infections. Therefore, it is necessary to carefully monitor the concentration of TNF-alpha (total and free), Humira, and if patients are making antibodies to the drug (Human anti-Humira antibodies).

ADI has developed new ELISA kits that measure **TNF-alpha, Free Humira (TNF-unbound)** in patients treated with Humira. ADI has also developed ELISA kits to detect antibodies to Humira (**Human Anti-Humira Antibodies**) in patients receiving long-term treatments. These kits will allow to research better ways to monitor humira treatment. Additional ELISA kits are available to monitor the increase other autoimmune diseases (ANA, anti-dsDNA IgGs, tuberculosis).

Catalog#	Product Description	Features
200-300-ADG	Humira/Adalimumab (Human Anti-TNF-alpha) ELISA Kit for dog, 96 tests	Range 0-250 ng/ml; Sensitivity ~1 ng/ml Samples (100 μ l; 1:10000, serum/plasma Time: 105 min assay (60+30+15) at room temp
200-310-AHG	Humira/Adalimumab (Human Anti-TNF-alpha) ELISA Kit for human, 96 tests	Range 0-50 ng/ml; Sensitivity ~0.25 ng/ml Samples (100 μ l; 1:10-1:100, serum/plasma Time: 105 min assay (60+30+15) at room temp
200-320-AHG	Human Anti-Humira/Adalimumab (Human Anti-TNF-alpha) ELISA Kit for human, 96 tests	Range 0-50 IU/ml; Sensitivity ~3 ng/ml Samples (100 μ l; 1:10-1:100, serum/plasma Time: 105 min assay (60+30+15) at room temp
200-330-TNF	Human TNF-alpha ELISA Kit, 96 tests	Range 0-200 pg/ml; Sensitivity ~10 pg/ml Samples (100 μ l; serum/plasma Time: 135 min assay (60+30+30+15) at room temp

ADI is also offering custom testing of animal or human samples for humira or antibodies to humira, and TNF alpha measurements.