Human IFNgamma ELISA

Cat.n° 31673539U1

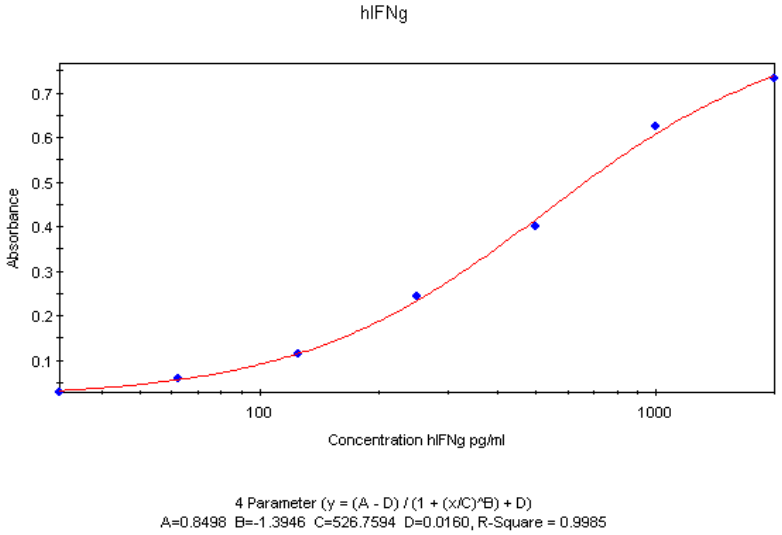
This ImmunoTools ELISA for quantification of natural and recombinant human **IFNgamma (hIFNg)** in cell culture supernatants and body fluids contains appropriate reagents sufficient for processing of 1 microplate (1 x 96 wells; 100 µl/well)

Typical standard curve range: 31 – 2000 pg/ml

Detection limit under optimal conditions: 24 pg/ml

Content:
1x vial lyophilized anti-human IFNgamma Capture-Antibody (red cap)
1x vial lyophilized anti-human IFNgamma Detector-Antibody (yellow cap)
1x vial lyophilized recombinant human IFNgamma standard (**50 ng rh IFNg**) (white cap)
1x vial liquid Poly-HRP-Streptavidin-HS (blue cap)
Spin down all vials before use

Additional material required:
96well-Microplates
Wash-Buffer (e.g. PBS + 0.05% Tween20)
Coating-Buffer (e.g. PBS)
Blocking-Buffer / Reagent-Diluent (e.g. PBS + 2% BSA + 0.05% Tween20)
Stop-Solution (e.g. 2 M H_2SO_4)
TMB-Solution



Note:
All steps of incubation except HRP-Streptavidin and TMB substrate can be carried out over-night at 2 – 8° C.
Do not use solutions containing sodium azide, nor add sodium azide to the supplied reagents. Sodium azide inactivates the peroxidase.

Storage:
Protect from light!
Store at 2-8° C or longterm storage at -20° C.
Reconstituted reagents should be stored at -20° C. Please prevent repeated freeze-thaw cycles.
The performance of the reagents is guaranteed until the expiration date shown on the label.

For research only. Not for use in diagnostic or therapeutic procedures.

ImmunoTools **Excellent Quality - Advantageously priced**
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Human IFNg-ELISA-procedure:

Coating
Reconstitute the lyophilized capture-antibody in 500 µl PBS.
Dilute capture-antibody 1:100 in coating-buffer (100 µl capture-antibody in 10 ml PBS).
Subsequently transfer 100 µl of this working-solution to each well and incubate overnight at room temperature.
↓
Remove capture-antibody completely
↓
Blocking
Transfer 300 µl blocking-buffer to each well and incubate 1 h at room temperature
↓
Remove Blocking-buffer completely
↓
Addition of standard & sample
Dilute standard & samples in reagent-diluent and transfer 100 µl of each mixture in the respective wells in duplicates.
Standard: Make serial dilutions in reagent-diluent and begin with a high standard and end with blanks.
The standard vial of this set contains **50 ng lyophilized rhIFNg**. Reconstitute this in exactly 1 ml reagent-diluent (stock solution = 50 ng/ml) and choose a sufficient high standard working solution for your assay.
Incubate at room temperature for 2 h.
↓
Wash 5x with washing-buffer
↓
Addition of biotinylated detector-antibody
Reconstitute the lyophilized detector-antibody in 500 µl blocking-buffer.
Dilute detector-antibody 1:100 in reagent-diluent (100 µl detector-antibody in 10 ml reagent-diluent).
Subsequently transfer 100 µl of this working-solution to each well and incubate 2 h at room temperature
↓
Wash 5x with washing-buffer
↓
Addition of Poly-HRP-Streptavidin-HS
Dilute Poly-HRP-Streptavidin-HS 1:1000 in reagent-diluent (10 µl in 10 ml reagent-diluent).
Subsequently transfer 100 µl of this working-solution to each well an incubate 30 min at room temperature.
↓
Wash 5x with washing-buffer
↓
Addition of TMB substrate
Warm the solution to room temperature before use.
Add 100 µl of the TMB to each well and incubate at room temperature up to 60 minutes*
When the enzymatic colour reaction is sufficiently proceeded stop the reaction by adding of 50 µl stop solution
↓
Read the microplate at **450** nm
(if wavelength correction is available, set to 540 nm, 570 nm or 630 nm as reference)

*The speed of enzymatic colour development is influenced by many customer-specific factors. Therefore the incubation time is variable und specific for each test system. The development of the colour reaction has to be controlled and should be stopped at an appropriate time point.

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