

Separate antibody reagents

Monoclonal antibodies



Product code	Product description	Quantity
3420-3-250	anti-human IFN- γ mAb 1-D1K, purified	250 μ g
3420-3-1000	anti-human IFN- γ mAb 1-D1K, purified	1 mg
3420-6-250	anti-human IFN- γ mAb 7-B6-1, biotinylated	250 μ g
3420-6-1000	anti-human IFN- γ mAb 7-B6-1, biotinylated	1 mg
3420-7	anti-human IFN- γ mAb 1-D1K, FITC	250 μ g

Clones

1-D1K, Mouse IgG1 (capture mAb)
7-B6-1, Mouse IgG1 (detection mAb)

Specificity

Native and recombinant human IFN- γ and native IFN- γ from chimpanzee. The two mAbs recognize different epitopes of IFN- γ .

Immunogen

Recombinant human IFN- γ was used as immunogen for the production of both mAbs.

Production and purification

Antibodies have been purified from *in vitro* cultures by protein G affinity chromatography.

Concentrations

The mAbs have been sterile filtered (0.22 μ m) and are delivered at 1 mg/ml in phosphate buffered saline with 0.02 % sodium azide.

Storage

Store product at 4-8°C or frozen at -20°C or below. Avoid repeated freezing and thawing.

Applications

Enumeration of IFN- γ -producing cells using ELISpot. Quantification of IFN- γ in solution using ELISA. MAb 1-D1K is recommended for capture and mAb 7-B6-1 for detection. Immunostaining of IFN- γ in immunofluorescence. MAb 1-D1K is suitable for flow cytometry.

Human IFN- γ antibodies

ELISpot^{PRO} kit



The ELISpot^{PRO} kit excludes the need for the coating step and also reduces the number of incubation steps. It contains plates precoated with capture mAb, detection antibody conjugated with ALP or HRP enzyme, substrate and a positive control (CD3-2).

Contents: • precoated PVDF plates or strip-plates • ALP- or HRP-labeled detection mAb
• BCIP/NBT or TMB substrate • positive control (mAb CD3-2)

Product code	Product description	Plate	mAb-Enzyme	Quantity
3420-2APT-2	h- IFN- γ ELISpot ^{PRO} kit	transparent	ALP	2 plates
3420-2APT-10	h- IFN- γ ELISpot ^{PRO} kit	transparent	ALP	10 plates
3420-2APW-2	h- IFN- γ ELISpot ^{PRO} kit	white	ALP	2 plates
3420-2APW-10	h- IFN- γ ELISpot ^{PRO} kit	white	ALP	10 plates
3420-2HPT-2	h- IFN- γ ELISpot ^{PRO} kit	transparent	HRP	2 plates
3420-2HPT-10	h- IFN- γ ELISpot ^{PRO} kit	transparent	HRP	10 plates
3420-2HPW-2	h- IFN- γ ELISpot ^{PRO} kit	white	HRP	2 plates
3420-2HPW-10	h- IFN- γ ELISpot ^{PRO} kit	white	HRP	10 plates
3420-2AST-2	h- IFN- γ ELISpot ^{PRO} kit	transparent	ALP	2 strip-plates

Precoated PVDF plates or strip-plates

Precoated PVDF (polyvinylidene fluoride) membrane plates are coated with capture mAb 1-D1K and should be stored at 4-8°C or at room temperature.

One-step detection reagent

The enzyme-labelled detection mAb, 7-B6-1-ALP or 7-B6-1-HRP, supplied in the ELISpot^{PRO} kit should be stored at 4-8°C.

Substrate

BCIP/NBT and TMB are supplied as ready-to-use solution and should be stored at 4-8°C.

Positive control

The mAb CD3-2 should be stored at 4-8°C.

Human IFN- γ ELISpot

ELISpot^{PLUS} kit



The ELISpot^{PLUS} kit contains PVDF plates, matched quantities of capture antibody, biotinylated detection antibody, Streptavidin-enzyme conjugate (ALP or HRP), substrate (BCIP/NBT or TMB) and a positive control (CD3-2). The kit is available with regular or precoated plates and precoated strip-plates.

- Contents:
- PVDF plates
 - capture mAb
 - biotinylated detection mAb
 - Streptavidin-enzyme (ALP or HRP)
 - BCIP/NBT or TMB substrate
 - positive control (CD3-2)

Product code	Product description	SA-Enzyme	Quantity
Regular plates:			
3420-2AW-Plus	h- IFN- γ ELISpot ^{PLUS} kit	ALP	4 white plates
3420-2HW-Plus	h- IFN- γ ELISpot ^{PLUS} kit	HRP	4 white plates
Precoated plates:			
3420-4APT-4	h- IFN- γ ELISpot ^{PLUS} kit	ALP	4 transp. plates
3420-4APW-4	h- IFN- γ ELISpot ^{PLUS} kit	ALP	4 white plates
3420-4HPT-4	h- IFN- γ ELISpot ^{PLUS} kit	HRP	4 transp. plates
3420-4HPW-4	h- IFN- γ ELISpot ^{PLUS} kit	HRP	4 white plates
Precoated strip-plates:			
3420-4AST-4	h- IFN- γ ELISpot ^{PLUS} kit	ALP	4 transp. plates
3420-4HST-4	h- IFN- γ ELISpot ^{PLUS} kit	HRP	4 transp. plates

PVDF plates

PVDF (polyvinylidene fluoride) membrane plates included in the ELISpot^{PLUS} kit should be stored at room temperature.

Precoated PVDF plates and precoated PVDF strip-plates

Precoated PVDF (polyvinylidene fluoride) membrane plates and precoated strip-plates are coated with capture mAb 1-D1K and should be stored at 4-8°C or at room temperature.

Antibodies

See section **Monoclonal antibodies**.

Streptavidin-enzyme conjugate

Secondary detection reagents, Streptavidin-ALP and -HRP, should be stored at 4-8°C.

Substrates

BCIP/NBT and TMB substrate are supplied as ready-to-use solutions and should be stored at 4-8°C.

Human IFN- γ ELISpot

ELISpot kit



The basic kit contains matched quantities of coating and detection reagents for ≥ 4 plates. This kit does not include ELISpot plates and substrate.

Contents: • capture mAb • biotinylated detection mAb • Streptavidin-enzyme (ALP or HRP)

Product code	Product description	Streptavidin-enzyme	Quantity
3420-2A	human IFN- γ ELISpot kit	ALP	for 4 plates*
3420-2H	human IFN- γ ELISpot kit	HRP	for 4 plates*

*sufficient for 4 plates when using recommended coating conc. of 15 $\mu\text{g}/\text{ml}$ of capture antibody.

Antibodies

See section **Monoclonal antibodies**.

Streptavidin-enzyme conjugate

Secondary detection reagents, Streptavidin-ALP and -HRP, should be stored at 4-8°C.

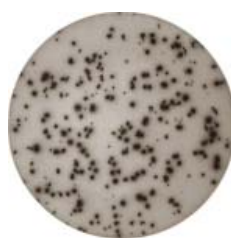
Typical images of human IFN- γ ELISpot wells:



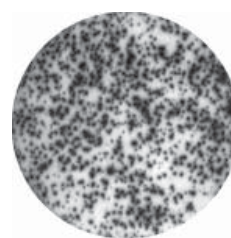
PPD
250 000 cells



No stimuli
50 000 cells



CEF peptide pool
250 000 cells



CD3 stimulation (CD3-2)
50 000 cells

IFN- γ response to antigen. Human PBMC were stimulated with Purified Protein Derivate (PPD) or cultured in medium alone (no stimuli) for 16 hours. The CEF peptide pool and CD3 stimulation using the CD3-2 mAb were used as positive controls.

ELISA^{PRO} kit



The ELISA^{PRO} kit is a complete kit based on precoated strip-plates and includes all reagents and buffers required for an easy and straight-forward assay. Careful validations have established these kits as highly sensitive tools for quantification of cytokines in both serum/plasma samples and in cell supernatants. The cytokine standard has been calibrated against an international reference reagent from NIAID (National Institute of Allergy and Infectious Diseases).

Contents: • precoated strip-plates • biotinylated detection mAb • Streptavidin-HRP
• cytokine standard • standard reconstitution buffer • wash buffer
• ELISA diluent • Streptavidin-HRP diluent • TMB substrate
• stop solution • adhesive plate covers

Product code	Product description	Quantity
3420-1HP-2	human IFN- γ ELISA ^{PRO} kit	2 plates
3420-1HP-10	human IFN- γ ELISA ^{PRO} kit	10 plates

Antibodies

See section **Monoclonal antibodies**.

Standard

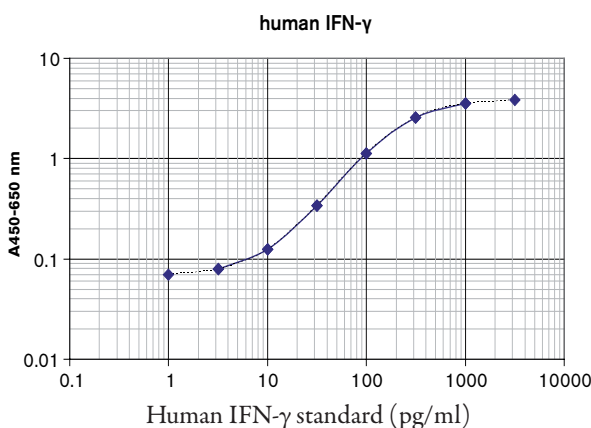
ELISA kits include a lyophilized recombinant human IFN- γ standard that should be stored at 4-8 °C. After reconstitution of the lyophilized standard, it should be aliquoted and kept at -20 °C.

Streptavidin-HRP conjugate

Secondary detection reagents, Streptavidin-HRP, should be stored at 4-8°C

Buffers, diluents, substrate, stop solution

All these components should be stored at 4-8 °C. The expiry date for the unopened kit is indicated on the box. An opened kit has an expiry date of 1 month if stored according to instructions.



Representative titration of human recombinant IFN- γ standard using an ELISA^{PRO} kit from Mabtech. The standard range is 3.16-1000 pg/ml.

Human IFN- γ ELISA

ELISA kit



The ELISA kits contain matched quantities of capture mAb, detection reagents and recombinant cytokine standard for 6 or 20 plates. The cytokine standard has been calibrated against an international reference reagent from NIAID (National Institute of Allergy and Infectious Diseases).

Contents: • capture mAb • biotinylated detection mAb

Product code	Product description	Streptavidin-enzyme	Quantity
3420-1A-6	human IFN- γ ELISA	ALP	for 6 plates
3420-1A-20	human IFN- γ ELISA	ALP	for 20 plates
3420-1H-6	human IFN- γ ELISA	HRP	for 6 plates
3420-1H-20	human IFN- γ ELISA	HRP	for 20 plates

Antibodies

See section **Separate antibody reagents**.

Streptavidin-enzyme conjugate

Secondary detection reagents, Streptavidin-ALP and -HRP, should be stored at 4-8°C.

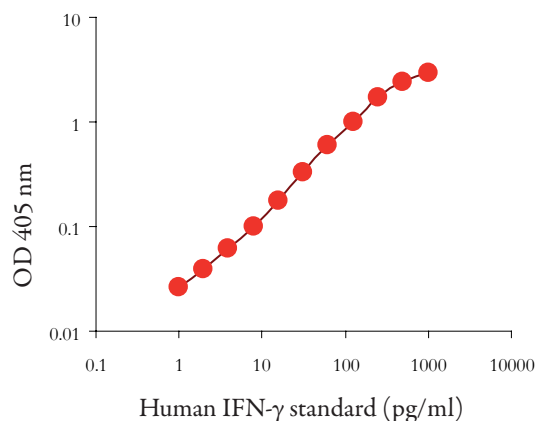
Standard

ELISA kits include a lyophilized recombinant human IFN- γ standard that should be stored at 4-8°C until aliquoted.

Note:

Mabtech offers an ELISA diluent particularly recommended for the dilution of serum/plasma samples. The diluent prevents false positive read-outs which may be caused by interference of heterophilic antibodies found in plasma and serum and is available separately. Please inquire.

Representative titration of human recombinant IFN- γ standard using an ELISA kit from Mabtech. The standard range is 4-400 pg/ml.



Human IFN- γ ELISA

References

Selected references using IFN- γ reagents from Mabtech

Human IFN- γ ELISpot^{PRO}

- Liebeschuetz S, *et al.* Diagnosis of tuberculosis in South African children with a T-cell- based assay: a prospective cohort study. *Lancet* 364: 2196, 2004.
- Liu XQ, *et al.* Evaluation of T-cell Responses to Novel RD1- and RD2-Encoded Mycobacterium tuberculosis Gene Products for Specific detection of Human Tuberculosis Infection. *Infect. Immun.* 72: 2574, 2004.
- Ewer K, *et al.* Comparison of T-cell-based assay with tuberculin skin test for diagnosis of Mycobacterium tuberculosis infection in a school tuberculosis outbreak. *Lancet* 361: 1168, 2003.

Human IFN- γ ELISpot

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- Burton CT, *et al.* Rapid qualitative and quantitative analysis of T-cell responses in HIV-1-infected individuals receiving successful HAART and HIV-1 seronegative controls: concomitant assessment of perforin, IFN-gamma and IL-4 secretion. *J. Immunol. Methods* 308: 216, 2006.
- Andersen MH, *et al.* Spontaneous Immunity against Bcl- x_L in Cancer Patients. *J. Immunol.* 175: 2709, 2005.
- Sacre K, *et al.* Repertoire, diversity, and differentiation of specific CD8 T cells are associated with immune protection against human cytomegalovirus disease. *J. Exp. Med.* 201: 1999, 2005.
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- Schuler-Turner B, *et al.* Rapid induction of tumor-specific type 1 T helper cells in metastatic melanoma patients by vaccination with mature, cryopreserved, peptide-loaded monocyte-derived dendritic cells. *J. Exp. Med.* 195: 1279, 2002.
- Makedonas G, *et al.* HIV-specific CD8 T-cell activity in uninfected injection drug users is associated with maintenance of sero-negativity. *AIDS* 16: 1595, 2002.
- Larsson M, *et al.* Amplification of low-frequency antiviral CD8⁺ type 1 responses using autologous dendritic cells. *AIDS* 16: 171, 2002.
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- Czerkinsky C, *et al.* Reverse ELISpot assay for clonal analysis of cytokine production. I. Enumeration of gamma-Interferon secreting cells. *J. Immunol. Methods* 110: 29, 1988.

Human IFN- γ ELISA

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- Passoni L, *et al.* ALK as a novel lymphoma-associated tumorantigen: identification of 2 HLA-A2.1-restricted CD8⁺ T-cell epitopes. *Blood* 99: 2100, 2002.

Monkey (chimpanzee) using human IFN- γ ELISpot:

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- Schneider J, *et al.* A prime-boost immunisation regimen using DNA followed by recombinant modified vaccinia virus Ankara induces strong cellular immune responses against the Plasmodium falciparum TRAP antigen in chimpanzees. *Vaccine* 19: 4595, 2001.



All our products are developed and manufactured by MABTECH AB, Sweden, whose quality management system complies with the following standards:

