abcam

Product datasheet

Recombinant Human Interferon gamma protein (Active) ab259377

9 Images

Description

Product name	Recombinant Human Interferon gamma protein (Active)
Biological activity	Fully biologically active determined by the dose dependent reduction in cytopathic effect of viral infection in A549 cells. ED_{50} for this effect is 0.30 ng/ml corresponding to a specific activity of 3.33×10^6 units/mg.
Purity	>= 95 % SDS-PAGE. >= 95 % HPLC.
Endotoxin level	< 0.005 Eu/µg
Expression system	HEK 293 cells
Accession	P01579
Protein length	Full length protein
Animal free	Yes
Carrier free	Yes
Nature	Recombinant
Species	Human
Sequence	QDPYVQEAENLKKYFNAGHSDVADNGTLFLGILKNWKEE SDRKIMQSQIV SFYFKLFKNFKDDQSIQKSVETIKEDMNVKFFNSNKKKRD DFEKLTNYSV TDLNVQRKAIHELIQVMAELSPAAKTGKRKRSQMLFRGRR ASQ
Predicted molecular weight	17 kDa
Amino acids	24 to 166
Additional sequence information	Full length protein including propeptide with a single glycine at the N-terminus

Specifications

Our **Abpromise guarantee** covers the use of **ab259377** in the following tested applications.

The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Applications	Mass Spectrometry

	Cell Culture
	HPLC
	SDS-PAGE
	Functional Studies
Form	Lyophilized
Additional notes	This protein is filter sterilised prior to aliquoting and lyophilisation. All aliquoting and lyophilisation steps are performed in a sterile environment

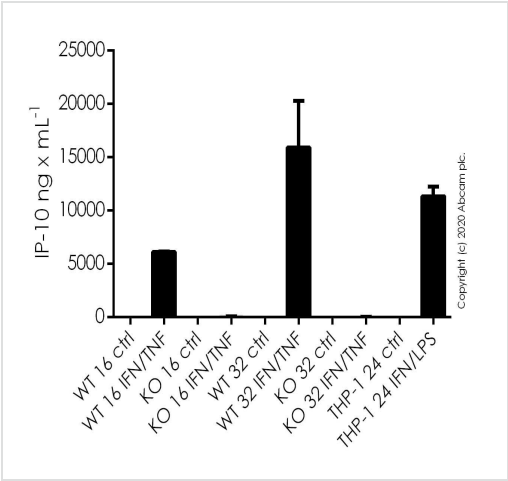
Preparation and Storage

Stability and Storage	Shipped at Room Temperature. Store at Room Temperature.
	pH: 6.00 Constituents: 0.727% Dibasic monohydrogen potassium phosphate, 0.248% Monobasic dihydrogen potassium phosphate, 10.26% Trehalose
	Buffer lyophilized from.
	This product is an active protein and may elicit a biological response in vivo, handle with caution.
Reconstitution	Reconstitute with Phosphate Buffered Saline.Reconstituted protein stable at -80C for 12 months or 4C for 1 week. Lyophilized contents may appear as either a translucent film or a white powder. This variance does not affect the quality of the product.

General Info

Function	Produced by lymphocytes activated by specific antigens or mitogens. IFN-gamma, in addition to having antiviral activity, has important immunoregulatory functions. It is a potent activator of macrophages, it has antiproliferative effects on transformed cells and it can potentiate the antiviral and antitumor effects of the type I interferons.
Tissue specificity	Released primarily from activated T lymphocytes.
Involvement in disease	In Caucasians, genetic variation in IFNG is associated with the risk of aplastic anemia (AA) [MIM:609135]. AA is a rare disease in which the reduction of the circulating blood cells results from damage to the stem cell pool in bone marrow. In most patients, the stem cell lesion is caused by an autoimmune attack. T-lymphocytes, activated by an endogenous or exogenous, and most often unknown antigenic stimulus, secrete cytokines, including IFN-gamma, which would in turn be able to suppress hematopoiesis.
Sequence similarities	Belongs to the type II (or gamma) interferon family.
Post-translational modifications	Proteolytic processing produces C-terminal heterogeneity, with proteins ending alternatively at Gly-150, Met-157 or Gly-161.
Cellular localization	Secreted.

Images

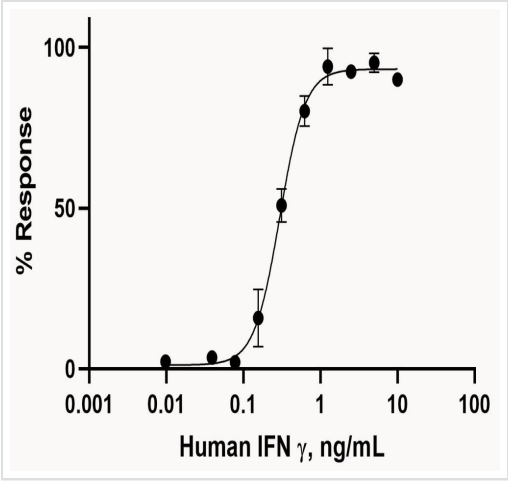


Sandwich ELISA - Recombinant Human Interferon gamma protein (Active) (ab259377)

Wild-type A549 control cells or IP-10 knockout A549 cells (**ab266969**), grown to 40% confluency, were stimulated with Recombinant Human Interferon gamma protein (ab259377) at 100 ng/ml and Recombinant human TNF alpha protein (**ab259410**) at 10 ng/ml or vehicle control for 16 or 32 hours.

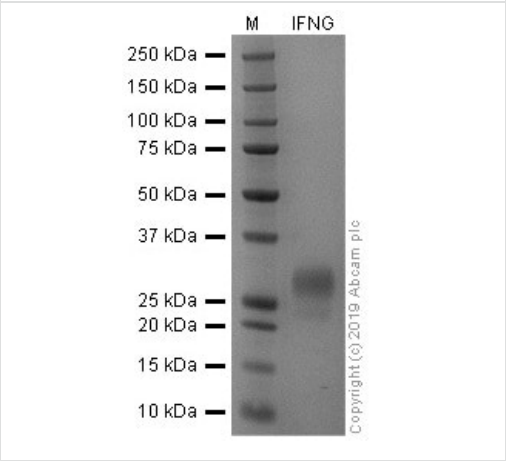
THP-1 cells, grown to 40% confluency, were stimulated with Recombinant Human Interferon gamma protein (ab259377) at 200 ng/ml and LPS at 50 ng/mL or vehicle control for 24 hours.

The concentrations of IP-10 (CXCL10) in cell culture supernatants were measured in duplicate and interpolated from the IP-10 standard curves using Human IP-10 ELISA Kit (**ab173194**). IP-10 from vehicle control samples were measured in undiluted supernatants and the treated samples were diluted 200 times. The interpolated dilution factor corrected values are plotted (mean +/- SD, n=2).



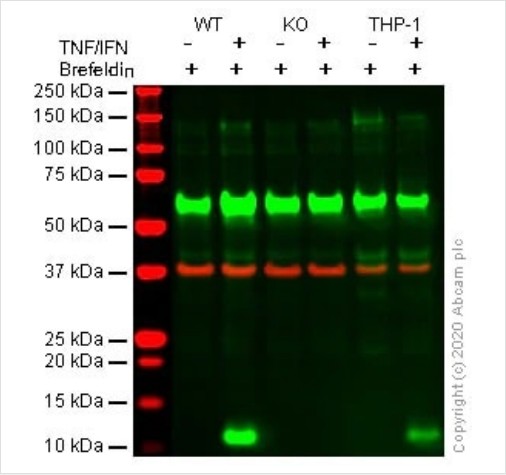
Functional Studies - Recombinant Human Interferon gamma protein (Active) (ab259377)

Fully biologically active determined by the dose dependent reduction in cytopathic effect of viral infection in A549 cells. ED_{50} for this effect is 0.30 ng/ml corresponding to a specific activity of 3.33×10^6 units/mg.



SDS-PAGE - Recombinant human Interferon gamma protein (Active) (ab259377)

SDS-PAGE analysis of ab259377.



Western blot - Recombinant Human Interferon gamma protein (Active) (ab259377)

All lanes : Anti-IP10 antibody [EPR20764] (**ab214668**) at 1/1000 dilution

Lane 1 : Wild-type A549 Brefeldin A (**ab120299**)-treated (5ug/ml, 6h) cell lysate
Lane 2 : Wild-type A549 IFN-y (ab259377) (100 ng/ml, 32 h) and TNF-alpha (**ab259410**) (10 ng/ml, 32h), and Brefeldin A (**ab120299**)-treated (5ug/ml for the last 6h) cell lysate
Lane 3 : IP10 knockout A549 Brefeldin A (**ab120299**)-treated (5ug/ml, 6h) cell lysate
Lane 4 : IP10 knockout A549 IFN-y (ab259377) (100ng/ml, 32h) and TNF-alpha (**ab259410**) (10ng/ml, 32h), and Brefeldin A (**ab120299**)-treated (5ug/ml for the last 6h) cell lysate
Lane 5 : THP-1 Brefeldin A (**ab120299**)-treated (5ug/ml, 6h) cell lysate
Lane 6 : THP-1 IFN-y (ab259377) (200ng/ml, 24h) and LPS (50ng/ml, 24h)-treated for 24 hours, and Brefeldin A (**ab120299**)-treated (5ug/ml for the last 6h) cell lysate

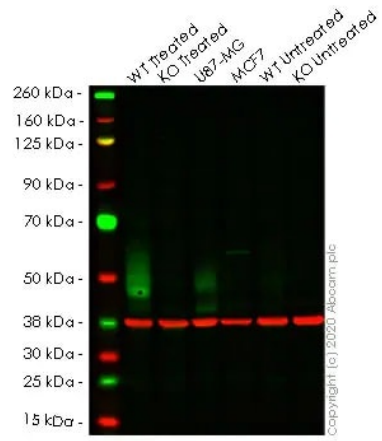
Lysates/proteins at 30 µg per lane.

Performed under reducing conditions.

Observed band size: 11 kDa

Lanes 1 - 6: Merged signal (red and green). Green - **ab214668** observed at 11 kDa. Red - loading control **ab8245** (Mouse anti-GAPDH antibody [6C5]) observed at 37kDa.

ab214668 was shown to react with IP10 in wild-type A549 cells in western blot with loss of signal observed in IP10 knockout cell line **ab266971** (knockout cell lysate **ab256888**). Wild-type and IP10 knockout A549 cell lysates were subjected to SDS-PAGE. Membranes were blocked in fluorescent western blot (TBS-based) blocking solution before incubation with **ab214668** and **ab8245** (Mouse anti-GAPDH antibody [6C5]) overnight at 4°C at a 1 in 1000 dilution and a 1 in 20000 dilution respectively. Blots were incubated with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preabsorbed (**ab216773**) and Goat anti-Mouse IgG H&L (IRDye® 800CW) preabsorbed (**ab216772**) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.



Western blot - Recombinant Human Interferon gamma protein (Active) (ab259377)

All lanes : Anti-PD-L1 antibody [EPR19759] (**ab213524**) at 1/1000 dilution

Lane 1 : Wild-type A549 treated with 100 ng/ml IFN gamma (ab259377) for 48 h cell lysate
Lanes 2 & 6 : CD274 knockout A549 treated with 100 ng/ml IFN gamma (ab259377) for 48 h cell lysate
Lane 3 : U-87 MG cell lysate
Lane 4 : MCF7 cell lysate
Lane 5 : Wild-type A549 untreated cell lysate

Lysates/proteins at 20 µg per lane.

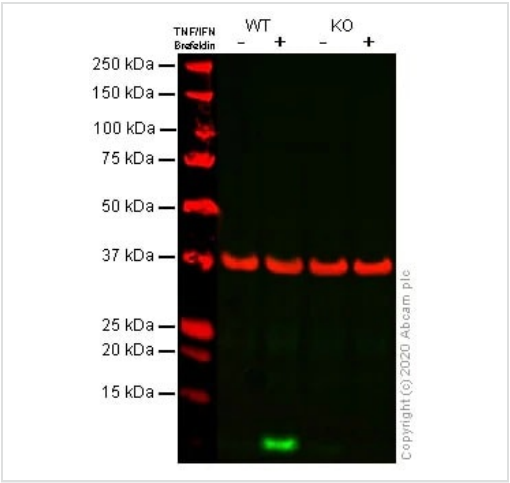
Performed under reducing conditions.

Observed band size: 50 kDa

Lanes 1- 6: Merged signal (red and green). Green - **ab213524** observed at 50 kDa. Red - Anti-GAPDH antibody [6C5] - Loading Control (**ab8245**) observed at 37 kDa.

ab213524 was shown to react with PD-L1 in wild-type A549 treated with 100 ng/ml IFN gamma for 48 h cells in western blot. Loss of signal was observed when both treated and untreated knockout cell lines **ab267054** (treated and untreated knockout cell lysates **ab256831**) were used. Wild-type A549 treated with 100 ng/ml IFN gamma for 48 h and CD274 knockout A549 treated with 100 ng/ml IFN gamma for 48 h cell lysates were subjected to SDS-PAGE. Membrane was blocked for 1 hour at room temperature in

0.1% TBST with 3% non-fat dried milk. **ab213524** and Anti-GAPDH antibody [6C5] - Loading Control (**ab8245**) were incubated overnight at 4°C at a 1 in 1000 dilution and a 1 in 20000 dilution respectively. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye®800CW) preadsorbed (**ab216773**) and Goat anti-Mouse IgG H&L (IRDye®680RD) preadsorbed (**ab216776**) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.



Western blot - Recombinant Human Interferon gamma protein (Active) (ab259377)

All lanes : Anti-IP10 antibody [EPR7850] (**ab137018**) at 1/500 dilution

Lane 1 : Wild-type A549 Brefeldin A (**ab120299**)-treated (5ug/ml, 6h) cell lysate
Lane 2 : Wild-type A549 IFN-y (ab259377) (100 ng/ml, 32 h) and TNF-alpha (**ab259410**) (10 ng/ml) for 32 hours, and Brefeldin A (**ab120299**)-treated (5ug/ml for the last 6h) cell lysate
Lane 3 : IP10 knockout A549 Brefeldin A (**ab120299**)-treated (5ug/ml, 6h) cell lysate
Lane 4 : IP10 knockout A549 IFN-y (ab259377) (100 ng/ml, 32 h) and TNF-alpha (**ab259410**) (10 ng/ml) for 32 hours, and Brefeldin A (**ab120299**)-treated (5ug/ml for the last 6h) cell lysate

Lysates/proteins at 30 µg per lane.

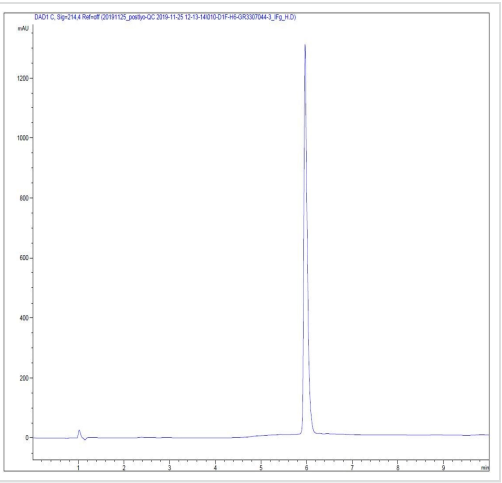
Performed under reducing conditions.

Observed band size: 11 kDa

Lanes 1 - 4: Merged signal (red and green). Green - **ab137018** observed at 11 kDa. Red - loading control **ab8245** (Mouse anti-GAPDH antibody [6C5]) observed at 37kDa.

ab137018 was shown to react with IP10 in A549 wild-type cells in western blot with loss of signal observed in IP10 knockout cell line **ab266969** (IP10 knockout cell lysate **ab256886**). A549 wild-type and IP10 knockout cell lysates were subjected to SDS-PAGE. Membranes were blocked in fluorescent western blot (TBS-based) blocking solution before incubation with **ab137018** and **ab8245** (Mouse anti-GAPDH antibody [6C5]) overnight at 4°C at a 1 in 500 dilution and a 1 in 20000 dilution respectively. Blots were incubated with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preabsorbed (**ab216773**) and Goat anti-Mouse IgG H&L (IRDye® 800CW)

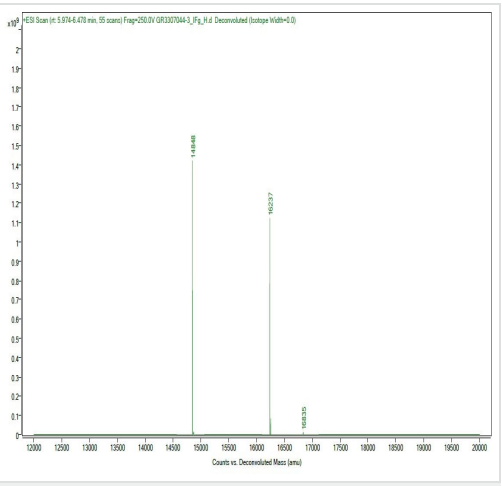
preabsorbed (**ab216772**) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.



HPLC - Recombinant human Interferon gamma protein (Active) (ab259377)

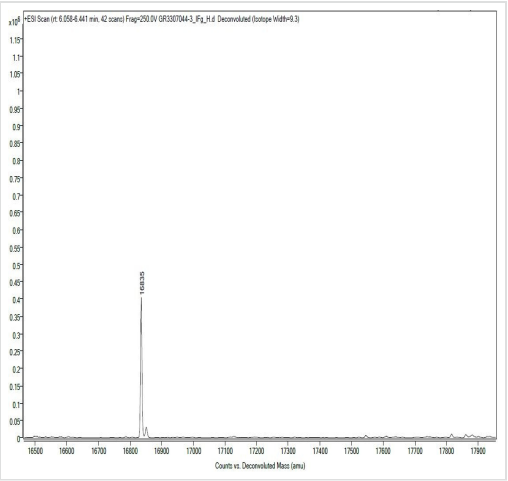
Purity: 100%

The spectrum was recorded using a 1260 Infinity II HPLC system with DAD (Agilent Technologies) and a MabPac RP column (3.0x100 mm, 4 µm, Thermo Scientific). 5 µL of purified protein was injected and the gradient run from 80 % water:TFA (99.9:0.1 v/v) and 20 % acetonitrile:water:TFA (90:9.9:0.1 v/v/v) to 20 % water:TFA (99.9:0.1 v/v) and 80 % acetonitrile:water:TFA (90:9.9:0.1 v/v/v) within 3 minutes followed by an isocratic step for another 3 min. Flow rate was 0.5 mL/min and the column compartment temperature was 50 °C.



Mass Spectrometry - Recombinant Human Interferon gamma protein (Active) (ab259377)

Mass Spectrometry analysis of ab259377 (Agilent Technologies). The mass spectrum shows the full length protein and two C-terminal truncated versions (-5 aa and -16 aa). Heterogeneity of C-terminus has been reported in literature (PAN, Yu‐Ching E., et al. "Structural characterization of human interferon γ Heterogeneity of the carboxyl terminus." European journal of biochemistry 166.1 (1987): 145-149.).



Mass Spectrometry - Recombinant human Interferon gamma protein (Active) (ab259377)

M + 1.4 Da (calc, mass 16833.6)

The spectrum was recorded with a 6545XT AdvanceBio LC/Q-TOF (Agilent Technologies) and a MabPac RP column (42.1x50 mm, 4 µm, Thermo Scientific). 5 µL of purified protein was injected and the gradient run from 85 % water:FA (99.9:0.1 v/v) and 15 % acetonitrile:FA (90:9.9:0.1 v/v/v) to 55 % water:FA (99.9:0.1 v/v) and 45 % acetonitrile:FA (90:9.9:0.1 v/v/v) within 3 minutes followed by an isocratic step for another 2.5 min. Flow rate was 0.4 mL/min and the column compartment temperature was 60 °C. Data was analysed and deconvoluted using the Bioconfirm software (Agilent Technologies).

Please note: All products are "FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES"

Our Abpromise to you: Quality guaranteed and expert technical support

- Replacement or refund for products not performing as stated on the datasheet
- Valid for 12 months from date of delivery
- Response to your inquiry within 24 hours
- We provide support in Chinese, English, French, German, Japanese and Spanish
- Extensive multi-media technical resources to help you
- We investigate all quality concerns to ensure our products perform to the highest standards

If the product does not perform as described on this datasheet, we will offer a refund or replacement. For full details of the Abpromise, please visit **https://www.abcam.com/abpromise** or contact our technical team.

Terms and conditions

- Guarantee only valid for products bought direct from Abcam or one of our authorized distributors