

# Anti-hIFN- $\alpha$ -IgG

Neutralizing IgG monoclonal antibody to human interferon alpha 2

Catalog code: hifna-mab1-02

<https://www.invivogen.com/anti-hifna-igg>

For research use only

Version 22K18-AK

## PRODUCT INFORMATION

**Contents:** 200  $\mu$ g purified Anti-hIFN- $\alpha$ -IgG monoclonal antibody (mAb), provided azide-free and lyophilized

**Target:** Human interferon-alpha 2 (hIFN- $\alpha$ 2)

**Specificity:** Reacts with hIFN- $\alpha$ 1, hIFN- $\alpha$ 2, hIFN- $\alpha$ 5, hIFN- $\alpha$ 8, hIFN- $\alpha$ 14, hIFN- $\alpha$ 16, hIFN- $\alpha$ 17 and hIFN- $\alpha$ 21. Very weakly reacts with hIFN- $\alpha$ 4 and hIFN- $\alpha$ 10. Does not react with hIFN- $\alpha$ 6 or hIFN- $\alpha$ 7.

**Clone:** H7WM116

**Isotype:** Human IgG1, kappa

**Source:** Chinese hamster ovary (CHO) cells

**Formulation:** 0.2  $\mu$ m filtered solution in a sodium phosphate buffer with saccharose, glycine and stabilizing agents

**Purity:** Purified by affinity chromatography with protein G

**Tested applications:** Neutralization & blocking *in vitro*

### Antibody resuspension (0.1 mg/ml)

*Note:* Ensure you see the lyophilized pellet before resuspension.

Resuspend Anti-hIFN- $\alpha$ -IgG with sterile water:

Add 2 ml of sterile water per 200  $\mu$ g vial.

### Storage and stability

- Product is shipped at room temperature. Upon receipt, store lyophilized antibody at -20°C.
- Reconstituted antibody is stable for 1 month when stored at 4°C and for 1 year when stored at -20°C. Avoid repeated freeze-thaw cycles.

### Quality control

- The antibody has been validated for neutralization using cellular assays.
- The complete sequence of this antibody has been verified.
- The absence of bacterial contamination (e.g. lipoproteins and endotoxins) has been confirmed using HEK-Blue™ TLR2 and HEK-Blue™ TLR4 cells.

## BACKGROUND

Interferon-alpha (IFN- $\alpha$ ) is a type I interferon that has both anti-viral and immunomodulatory activities<sup>1</sup>. IFN- $\alpha$  is produced primarily by plasmacytoid dendritic cells<sup>2</sup>. Thirteen different human IFN- $\alpha$  subtypes have been described. Although the reason for the multiple IFN- $\alpha$  subforms is not fully understood<sup>3</sup>, IFN- $\alpha$  binds to a ubiquitously expressed heterodimeric receptor composed of two chains (IFNAR1 and IFNAR2), resulting in the recruitment of JAK1 and TyK2. These kinases phosphorylate STAT1 and STAT2, leading to the formation of the IFN-stimulated gene factor 3 (ISGF3) complex, which binds to IFN-stimulated response elements (ISRE), thereby directly activating the transcription of IFN-stimulated genes (ISGs)<sup>4</sup>.

1. Trinchieri G., 2010. Type I interferon: friend or foe? JEM 207(10):2053-2063.
2. Ivashkiv LB. & Donlin LT., 2014. Regulation of type I interferon responses. Nat Rev Immunol. 14(1):36-49.
3. Gibbert K. et al., 2013. IFN- $\alpha$  subtypes: distinct biological activities in anti-viral therapy. Br J Pharmacol. 168(5):1048-58.
4. Theofilopoulos A. et al., 2005. Type I interferons (alpha/beta) in immunity and autoimmunity. Annu Rev Immunol. 23:307-36.

## DESCRIPTION

Anti-hIFN- $\alpha$ -IgG is a recombinant mAb recognizing human interferon  $\alpha$  2 (hIFN- $\alpha$ 2). It has been selected for its ability to efficiently neutralize the biological activity of hIFN- $\alpha$ 2. This antibody reacts with various subtypes of the hIFN- $\alpha$  (see product information under specificity). Anti-hIFN- $\alpha$ -IgG (clone H7WM116) was generated by recombinant DNA technology. It has been produced in CHO cells and purified by affinity chromatography.

## APPLICATIONS

Anti-hIFN- $\alpha$ -IgG is a neutralizing antibody. It can be used to block hIFN- $\alpha$ -induced cellular activation *in vitro*, as described below.

## NEUTRALIZATION PROTOCOL

The exact concentration of antibody required to neutralize hIFN- $\alpha$  activity is dependent on the cytokine concentration, cell type and growth conditions. Below is a protocol using recombinant hIFN- $\alpha$ 2 as well as HEK-Blue™ IFN- $\alpha$ / $\beta$  cells. These cells are HEK293 cells stably expressing the human STAT2 and IRF9 genes, together with an IFN- $\alpha$ / $\beta$ -inducible SEAP (secreted embryonic alkaline phosphatase) reporter gene. Changes in SEAP activity in the supernatant due to inhibition of IFN- $\alpha$  receptor binding can be assessed using QUANTI-Blue™ Solution, a SEAP detection reagent.

In a 96-well plate:

1. Prepare a serial dilution of the Anti-hIFN- $\alpha$ -IgG or a negative control (e.g. Anti- $\beta$ -Gal-hlgG1) starting 10 ng/ml to 1  $\mu$ g/ml (final conc.).
2. Add 0.3 ng/ml of recombinant hIFN- $\alpha$ 2 to a final volume of 40  $\mu$ l.
3. Incubate for 30 minutes at 37°C, 5% CO<sub>2</sub>.
4. Prepare a suspension of HEK-Blue™ IFN- $\alpha$ / $\beta$  cells (~3.0 x 10<sup>5</sup> cells/ml) in culture medium.
5. Add 160  $\mu$ l (5 x 10<sup>4</sup> cells/well) of the cell suspension to each well
6. Incubate the plate at 37°C, 5% CO<sub>2</sub> for 24 hours.
7. The next day: prepare QUANTI-Blue™ Solution and carry out the measurements following the instructions on the data sheet.

## RELATED PRODUCTS

| Product                                 | Cat. Code    |
|---|--------------|
| Anti- $\beta$ -Gal-hlgG1                | bgal-mab1    |
| HEK-Blue™ IFN- $\alpha$ / $\beta$ Cells | hkb-ifnab    |
| QUANTI-Blue™ Solution                   | rep-qb-1     |
| Recombinant human IFN- $\alpha$ 2b      | rcyc-hifna2b |

### TECHNICAL SUPPORT

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