LumiKine™ hIFN-β

Human IFN-beta bioluminescent ELISA kit

Catalog # lumi-hifnb

For research use only

Version # 16H17-MM

PRODUCT INFORMATION

Contents (for five plates)

- 50 µg lyophilized hIFN-β Capture Antibody
- 5 µg lyophilized hIFN-β Biotinylated Antibody (detection antibody)
- 100 ng lyophilized hIFN-β Standard (CHO-expressed cytokine)
- 2 pouches of QUANTI-Luc™
- 1 vial Streptavidin-Lucia
- 1 white flat-bottom MaxiSorp® 96-well plate with a plate sealer

Storage and stability

- Products are shipped at room temperature. Store at -20 °C. Lyophilized products are stable for 12 months when properly stored.
- Resuspended antibodies are stable for 1 month when stored at 4 °C or 12 months when aliquoted and stored at -20 °C.
- Resuspended cytokine is stable for 1 month when stored at 4 °C or 12 months when aliquoted and stored at -80 °C.
- Resuspended Streptavidin-Lucia is stable for 6 months when aliquoted and stored at -20 °C.
- Reconstituted QUANTI-Luc™ is stable for 1 week at 4 °C or 1 month at -20 °C. Prepare aliquots and protect from light.

Note: Avoid repeated freeze-thaw cycles for all products stored at -20 °C or -80 °C.

Quality control

- The sensitivity and specificity of this kit are validated for each lot.
- Rigorous quality control tests are performed to ensure lot-to-lot reproducibility and performance.

DESCRIPTION

LumiKine™ hIFN-β is a bioluminescent ELISA kit designed to quantify the levels of human interferon-beta (hIFN-β) in cell culture supernatant, serum and plasma samples. This kit uses optimized matched pairs of monoclonal capture and detection antibodies to achieve sensitive and accurate measurement of the cytokine. The detection antibody is labeled with biotin to allow subsequent binding to streptavidin-Lucia, streptavidin conjugated to Lucia luciferase, a secreted reporter enzyme. Levels of hIFN-β are determined by measuring the luminescence produced by Lucia luciferase after hydrolysis of its substrate, coelenterazine. This substrate is provided in QUANTI-Luc™, a reagent that allows immediate reading of the luminescence.

Features

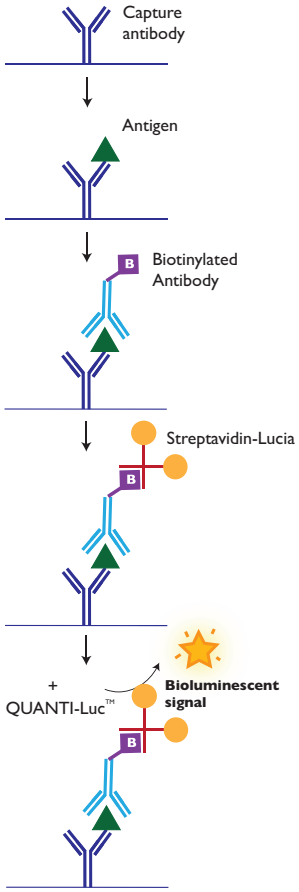
- **Target:** natural and recombinant human IFN-β
- **Standard:** CHO-expressed human IFN-β
- **Format:** bioluminescent ELISA in white immuno 96-well plate
- **Measurement:** relative light units (RLUs)
- **Incubation Time:** 4.5 hours
- **Limit of detection:** 32 pg/ml (for calculation method, see reference 4)
- **Specificity:** no cross-reactivity with mouse IFN-β, human or mouse IFN-α
- **Sample size and type:** 100 µl of cell culture supernatant, serum and plasma

PRINCIPLE

The monoclonal hIFN-β Capture Antibody is coated onto the wells of a white immuno 96-well plate. Samples and the hIFN-β Standard are pipetted into these wells, followed by the addition of the monoclonal Biotinylated Antibody (detection antibody).

During the first incubation, the hIFN-β antigen binds to the immobilized hIFN-β Capture Antibody on one site, and during the second incubation, to the Biotinylated Antibody on a second site.

After removal of the excess detection antibody, Streptavidin-Lucia is added. This streptavidin conjugate binds to the Biotinylated Antibody. After a short third incubation and washing to remove all the unbound Lucia luciferase, QUANTI-Luc™, a solution that contains the substrate of Lucia luciferase, is added. No further incubation is required. The bioluminescent signal is emitted instantaneously and measured in Relative Light Units (RLU) by a luminometer. The intensity of this signal is directly proportional to the concentration of hIFN-β present in the samples.



BACKGROUND

Interferon-beta (IFN-β) is a type I interferon that has both anti-viral and immunomodulatory activities[1]. IFN-β is a secreted polypeptide that is produced mainly by fibroblasts. In contrast to IFN-α, IFN–β is encoded by a single gene resulting in a single IFN–β isotype[3]. IFN-β 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)[3].

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. Theofilopoulos A. et al., 2005.** Type I interferons (alpha/beta) in immunity and autoimmunity. Annu Rev Immunol. 23:307-36. **4. Little TA., 2015.** Method Validation Essentials, Limit of Blank, Limit of Detection, and Limit of Quantitation. BioPharm International. 28:4.

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OTHER SOLUTIONS & MATERIALS REQUIRED

- Coating Buffer: 0.2 M carbonate/bicarbonate buffer pH 9.4, 0.2 µm filtered

Note: Alternatively, you can use sterile PBS.

- Blocking buffer: PBS containing 2% BSA and 0.05% Tween 20, 0.2 µm filtered
- Wash buffer: PBS containing 0.05% Tween 20
- Reagent Diluent: PBS containing 1% BSA and 0.05% Tween 20, 0.2 µm filtered

- Luminometer
- Plate sealers
- Clean paper towels
- White flat-bottom 96-well ELISA plates (e.g. Nunc LumiNunc™ MaxiSorp® plates)

METHODS

Reagent Preparation

Bring all reagents to room temperature before use. Allow all components to sit for a minimum of 15 minutes with gentle agitation after initial reconstitution. Working dilutions should be prepared and used immediately.

Capture Antibody stock solution (100 µg/ml)

- Add 500 µl of sterile PBS and mix by pipetting up and down until completely dissolved.
- Use immediately or prepare aliquots and store at -20 °C.
- Dilute with Coating Buffer to a working concentration of 1 µg/ml.

Biotinylated Antibody stock solution (10 µg/ml)

- Add 500 µl of sterile PBS and mix by pipetting up and down until completely dissolved.
- Use immediately or prepare aliquots and store at -20 °C.
- Dilute with Reagent Diluent to a working concentration of 50 ng/ml.

Standard stock solution (100 ng/ml)

- Add 1 ml of Reagent diluent and mix by pipetting up and down until completely dissolved.
- Use immediately or prepare aliquots and store at -80 °C.

QUANTI-Luc™ assay solution

- Pour the pouch contents into a 50 ml screw cap tube.
- Add 25 ml of sterile water.
- Swirl product gently until powder is completely dissolved.
- Use QUANTI-Luc™ assay solution immediately or store at -20 °C.

Note: This product is photosensitive and must be protected from light.

Streptavidin-Lucia stock solution

- Reconstitute product by adding 500 µl H_2O to the content of each tube.
- Mix by vortexing.
- Use immediately or prepare aliquots and store at -20 °C.

General ELISA Protocol

Plate Preparation

1. Add 100 µl of Capture Antibody (diluted to 1 µg/ml in Coating Buffer) to each well of a white flat-bottom MaxiSorp® 96-well plate. Cover the plate with an adhesive seal and incubate overnight at room temperature.
2. Remove excess Capture Antibody by flicking the plate over a sink and patting it against clean paper towels to remove any remaining drops.
3. Add 200 µl of Blocking Buffer to each well and incubate for 2 hours at 37 °C.
4. Remove Blocking buffer by flicking the plate over a sink and patting it against clean paper towels. The plate is now ready for sample addition. Alternatively, the plate can be covered with an adhesive seal and stored at -20 °C for 3 months.

Assay Procedure

Note: We suggest to test standards and samples in triplicate.

1. Add 100 µl of sample or standard diluted in Reagent Diluent, or appropriate diluent, per well. Cover with an adhesive seal and incubate for 2 hours at 37 °C.

Notes:

- The Reagent Diluent selected for use can alter the performance of the immunoassay. Optimization of the Reagent Diluent for samples with complex matrices, such as serum and plasma, may improve the performance of the assay.

- We recommend to plot a seven point standard curve using a 2-fold serial dilution with the highest standard at 1000 pg/ml.

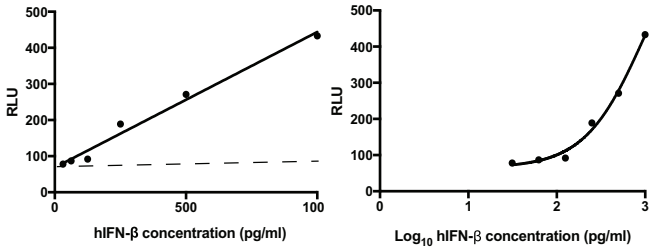
2. Remove the liquid by flicking the plate over a sink. Fill each well with 200 µl of Wash Buffer. Repeat the washing process twice for a total of three washes. After the last wash, remove any remaining Wash buffer by patting the plate against clean paper towels.
3. Add 100 µl of Biotinylated Antibody (diluted to 50 ng/ml in Reagent Diluent). Cover with an adhesive seal and incubate for 2 hours at 37 °C.
4. Repeat the washing process as in step 2.
5. Add 100 µl of Streptavidin-Lucia diluted in reagent Diluent (1:1000). Cover with an adhesive seal and incubate for 30 min at 37 °C.
6. During this incubation time, prepare the QUANTI-Luc™ assay solution. If frozen bring to 15-25 °C.
7. Repeat the washing process as in step 2.
8. Set luminometer reading time to the minimum value (0.1-0.5 second).
9. Add 50 µl of QUANTI-Luc™ to each well and proceed IMMEDIATELY with the measurement.

CALCULATION OF RESULTS

Create a standard curve using a computer software capable of generating a four parameter logistic (4-PL) curve fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph. The data may be linearlized by plotting the log of the cytokine concentrations versus the log of the relative light units (RLU) and the best fit line can be determined by regression analysis. This procedure will produce an adequate but less precise fit of the data.

TYPICAL DATA

This standard curve is only for demonstration purposes. A standard curve should be generated for each set of samples assayed. The dotted line represents the lower limit of detection.



RELATED PRODUCTS

Product	Catalog Code
QUANTI-Luc™	rep-qlc1
LumiKine™ mIFN-α	lumi-mifna
LumiKine™ Xpress hIFN-α	luex-hifna
LumiKine™ Xpress mIFN-β	luex-mifnb

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