LumiKine™ Xpress mIFN-α 2.0

Second generation murine IFN-alpha2 bioluminescent ELISA

Catalog code: luex-mifnav2

https://www.invivogen.com/lumikine-xpress-mifna

For research use only

Version 23A18-MM

PRODUCT INFORMATION

Contents (for five plates)

- 150 µg lyophilized mIFN-α capture antibody
- 5 µg lyophilized Lucia-conjugated detection antibody
- 100 ng lyophilized mIFN-α2 standard
- 2 tubes of QUANTI-Luc™ 4 Reagent, a Lucia luciferase detection reagent (sufficient to prepare 50 ml)
- 5 white flat-bottom MaxiSorp® 96-well plates and plate sealers

Storage and stability

- Products are shipped at room temperature.
- Store antibodies, cytokine and QUANTI-Luc™ 4 Reagent at -20 °C.
- Resuspended antibodies are stable for 1 month when stored at 4 °C or for 12 months when aliquoted and stored at -20 °C. Avoid repeated freeze-thaw cycles.
- Resuspended cytokine is stable for 1 month at 4 °C or for 12 months when aliquoted and stored at -80 °C. Avoid repeated freeze-thaw cycles.
- After preparation, QUANTI-Luc™ 4 Reagent working solution is stable for 48 hours at 4°C and for 1 month at -20°C. Prepare aliquots to avoid repeated freeze-thaw cycles. Protect from light.

Quality control

- The sensitivity of LumiKine™ Xpress mIFN-α 2.0 has been validated.

PRODUCT DESCRIPTION

LumiKine™ Xpress mIFN-α 2.0 is a bioluminescent ELISA kit designed to rapidly quantify the levels of murine interferon-alpha (mIFN-α) in cell culture supernatant, serum, and plasma samples. IFN-α is a type I IFN that has both anti-viral and immunostimulatory effects[1]. There are 17 different murine IFN-α subtypes (including 3 pseudogenes) which seemingly do not diverge in terms of biological function but instead most likely evolved to acquire specific expression patterns[2]. The binding of mIFN-α's to their receptor initiates a signaling cascade, ultimately resulting in the activation of IFN stimulated genes (ISGs)[1]. LumiKine™ Xpress mIFN-α 2.0 has been enhanced for both faster results and increased sensitivity. This kit uses an optimized pair of capture and detection antibodies. During a single 2-hour incubation, the mIFN-α antigen will bind to **1)** an immobilized mIFN-α capture antibody, as well as **2)** a detection antibody transcriptionally fused to Lucia luciferase; eliminating a step and saving time. Levels of mIFN-α in the sample are determined using QUANTI-Luc™ 4 Reagent, a Lucia luciferase detection reagent. The bioluminescent signal is emitted instantaneously and is assessed by luminometry. The intensity of this signal is directly proportional to the concentration of mIFN-α present in the samples.

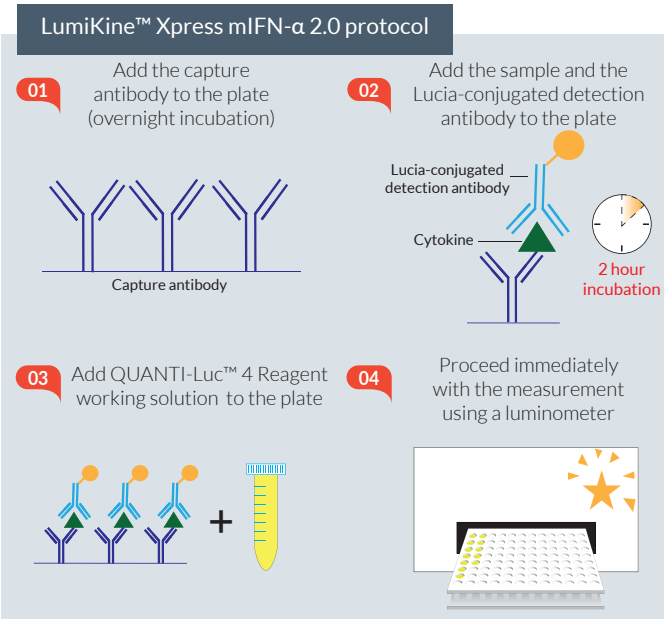
1. Schreiber G. 2017. The molecular basis for differential type I interferon signaling. J. Biol. Chem. 292:7285-94. **2. van Pesch V. *et al.*, 2004.** Characterization of the murine alpha interferon gene family. J. Virology. 78(15):8219-28.

KEY FEATURES

- **Incubation Time:** 2 hours (after plate preparation)
- **Limit of detection:** 7 pg/ml
- **Specificity:** No cross-reactivity with human (h)IFN-α2, hIFN-β, or mIFN-β
- **Target:** Natural and recombinant murine IFN-α2

Note: Other murine IFN-α subtypes may also be detected with variable limits of detection.

- **Standard cytokine:** HEK293-expressed mIFN-α2
- **Measurement:** Bioluminescent ELISA - relative light units (RLUs)
- **Sample size and type:** 50 µl of cell culture supernatant, serum, or plasma



METHODS

Solutions Required

Prepare the following solutions:

Note: All solutions should be filtered through a 0.2 µm filter before use.

- **Coating buffer**: 0.2M carbonate/bicarbonate buffer (pH 9.4),

Note: Alternatively, you can use sterile PBS (phosphate buffered saline).

- **Blocking buffer**: PBS containing 3% BSA and 0.05% Tween 20
- **Wash buffer**: PBS containing 0.05% Tween 20
- **Reagent diluent**: DMEM, 10% heat inactivated (HI)-FBS

Note: 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.

TECHNICAL SUPPORT
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Reagent Preparation

Note: 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.

a) mIFN-α capture antibody stock solution (300 µg/ml)

- Add 500 µl of sterile PBS to the vial and mix by pipetting until completely dissolved.
- Use immediately or prepare aliquots and store at -20 °C. Avoid repeated freeze-thaw cycles.
- Dilute with coating buffer (1/60) to a working concentration of 5 µg/ml.

b) Lucia-conjugated detection antibody stock solution (10 µg/ml)

- Add 500 µl of sterile PBS to the vial and mix by pipetting until completely dissolved.
- Use immediately or prepare aliquots and store at -20 °C. Avoid repeated freeze-thaw cycles.
- Dilute with reagent diluent to a working concentration of 30 ng/ml.

c) mIFN-α2 standard stock solution (100 ng/ml)

- Add 1 ml of PBS containing 1% BSA and 0.05% Tween 20 (0.2 µm filtered) to the vial, and mix by pipetting until completely dissolved.
- Use immediately (see 'General ELISA protocol' section (b)) or prepare aliquots and store at -80 °C. Avoid repeated freeze-thaw cycles.

d) QUANTI-Luc™ 4 Reagent

1. Dilute the total volume of the 20X tube (1.25 ml) of Reagent into 23.75 ml sterile water to obtain 25 ml of working solution.
2. Vortex very briefly (a few seconds).
3. Use the working solution immediately or store until required for use. The working solution is stable for 48 hours at 4°C and for 1 month at -20°C. Prepare aliquots to avoid repeated freeze-thaw cycles.

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

General ELISA Protocol

a) Plate Preparation

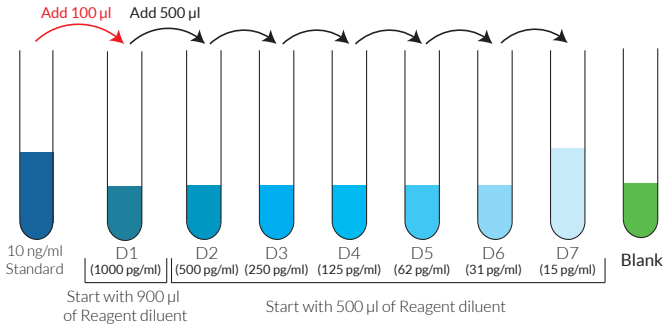
1. Add 50 µl of mIFN-α capture antibody (diluted to 5 µg/ml in coating buffer) to each well of a white flat-bottom MaxiSorp® 96-well plate.
2. Cover the plate with an adhesive seal and incubate overnight at room temperature.
3. Remove excess capture antibody by flicking the plate over a sink and patting it against clean paper towels to remove any remaining drops.
4. Add 200 µl of blocking buffer to each well and incubate for 2 hours at 37 °C.
5. 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.

b) General Standard Curve Setup

Below is an example protocol for setting up a 7-point standard curve using a 2-fold serial dilution with the highest standard at 1000 pg/ml.

1. To prepare a 10 ng/ml mIFN-α2 standard solution, add 30 µl of the standard stock solution (100 ng/ml) to 270 µl of reagent diluent.
2. Add 100 µl of the 10 ng/ml standard solution to 900 µl of reagent diluent. This is the first dilution (D1; 1000 pg/ml).
3. Add 500 µl of D1 to 500 µl of reagent diluent. This is the second dilution (D2).
4. Continue to perform a 2-fold serial dilution until D7.
5. Ensure you have a negative control/blank (reagent diluent only).

How to set up the 7-point standard curve:



c) Assay Procedure

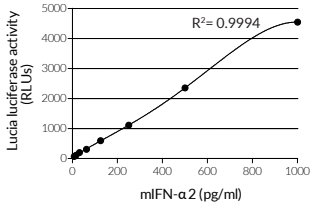
Note: We recommend to test all standards and samples in triplicate.

1. Add 50 µl of each sample (diluted in reagent diluent or an appropriate diluent) per well of the pre-coated plate.
2. Add 50 µl of each prepared standard (including a Blank) to additional wells.
3. Straight away add 50 µl of Lucia-conjugated detection antibody (diluted to 30 ng/ml in reagent diluent) to all wells.
4. Cover with an adhesive seal and incubate for 2 hours at 37 °C.
5. Prepare QUANTI-Luc™ 4 Reagent working solution (see 'Reagent preparation section (d)). If frozen bring to room temperature.
6. 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.
7. Set luminometer reading time to the minimum value (0.1-0.5 second).
8. Add 50 µl of QUANTI-Luc™ 4 Reagent working solution to each well and proceed IMMEDIATELY with the measurement.

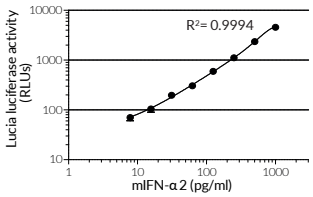
STANDARD CURVE DETERMINATION

Create a standard curve using computer software capable of generating a four parameter logistic (4-PL) curve fit. As an alternative, construct a standard curve by plotting the relative light units (RLUs) (triplicate data averaged) on the y-axis, and the different standard concentrations on the x-axis. Draw a best fit curve through as many points as possible on the graph. The data may be linearlized by plotting the log of the standard concentrations versus the log of the relative light units (RLUs) and the best fit line can be determined by a regression analysis. This procedure will produce an adequate but less precise fit of the data.

Typical Data



4-PL curve fit with standard axis



4-PL curve fit with $\log_{10}$ axis

These standard curves are only for demonstration purposes. A standard curve should be generated for each set of samples assayed.

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QUANTI-Luc™ 4 Reagent

A coelenterazine-based luminescence assay reagent

https://www.invivogen.com/quanti-luc

For research use only

Version 23A16-MM

PRODUCT INFORMATION

Contents

- 1 tube of **QUANTI-Luc™ 4 Reagent (20X)**

One tube of QUANTI-Luc™ 4 Reagent is sufficient for 5 x 96-well plates (25 ml standard Flash/end-point detection).

Note: This sample cannot be sold separately from the QUANTI-Luc™ 4 Lucia/Gaussia kit.

QUANTI-Luc™ 4 Lucia/Gaussia comprises two liquid components:

- QUANTI-Luc™ 4 Reagent 20X (coelenterazine substrate)
- QUANTI-Luc™ 4 Stabilizer 25X (optimized Glow assay reagent)

Find more information at https://www.invivogen.com/quanti-luc.

Storage and Stability

– Store QUANTI-Luc™ 4 Reagent at -20°C for up to 12 months.
– After preparation, the working solution is stable for 48 hours at 4°C and 1 month at -20°C. Prepare aliquots to avoid repeated freeze-thaw cycles.
Note: This product is photosensitive and should be protected from light.

Quality Control

Each lot is thoroughly tested to ensure the absence of lot-to-lot variation.
– Physicochemical characterization (pH, appearance).
– Functional assays using recombinant Lucia protein or reporter cells.

DESCRIPTION

QUANTI-Luc™ 4 Reagent is a component of the QUANTI-Luc™ 4 Lucia/Gaussia kit. It contains the coelenterazine substrate for the detection of secreted Lucia or Gaussia activity in live-cell supernatants, and of intracellular Renilla after cell lysis. The light signal produced correlates to the amount of luciferase protein expressed. It is quantified using a luminometer and expressed as relative light units (RLUs).

METHODS

Preparation of QUANTI-Luc™ 4 Reagent working solution (1X):

1. Dilute the total volume of the 20X tube (1.25 ml) of Reagent into 23.75 ml of sterile water to obtain 25 ml of working solution.
2. Vortex **very briefly** (a few seconds).
3. Use the working solution immediately or store until required for use. QUANTI-Luc™ 4 Reagent working solution can be stored for 48 hours at 4°C or 1 month at -20°C.

Flash detection of luciferase activity from cell culture medium:

To obtain **end-point readings** using a luminometer **with an injector**.
1. Set the luminometer with the following parameters: 50 µl of injection, end-point measurement with a 4 second start time and 0.1 second reading time.
2. Pipet 20 µl of sample per well into a 96-well white (opaque) or black plate, or a luminometer tube.
3. Prime the injector with QUANTI-Luc™ 4 Reagent 1X and proceed **immediately** with the measurement.

To obtain **end-point readings** using a luminometer **without injectors**.
1. Set the luminometer with a 0.1 second reading time.
2. Pipet 20 µl of sample per well into a 96-well white (opaque) or black plate, or a luminometer tube.
3. Add 50 µl of QUANTI-Luc™ 4 Reagent 1X to each well or tube.
4. Gently tap the plate several times to mix (do **not** vortex).
5. Proceed **immediately** with the measurement.

RELATED PRODUCTS

Product	Cat. Code
QUANTI-Luc™ 4 Lucia/Gaussia Kit	
500 tests	rep-qlc4lg1
2 x 500 tests	rep-qlc4lg2
5 x 500 tests	rep-qlc4lg5

TECHNICAL SUPPORT
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