Human IFN omega Matched Antibody Pair

Matched Antibody Pair for the development of an ELISA for quantitative detection of human IFN-ω

Catalog Number BMS233MST

Pub. No. MAN0016607 **Rev.** A.0 (30)

WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from **thermofisher.com/support**.

Read before opening

- Some vials contain small quantities of material, therefore centrifuge before use.
- This set of reagents is intended for use by persons experienced in the use of immunoassays. It is not suitable for use by inexperienced personnel.
- A sample protocol is included but please note that the protocol provided is a guideline. The type of substrate as well as all other reagents not included in the Module Set may influence assay performance.

Reagents provided

1 vial (1.1 mL) monoclonal Coating Antibody to human IFN-ω (100 µg/mL)

1 vial (55 µL) Biotin-Conjugate anti-human IFN-ω monoclonal antibody

1 vial (11 µL) Streptavidin-HRP

1 vial (30 µL) human IFN-ω Standard protein, 60 ng/mL

2 vials (50 mL) Sample Diluent

Storage instructions

Store kit components at -20°C. Immediately after use remaining reagents should be returned to -20°C storage, respectively. Avoid multiple freeze-thaw cycles. Aliquot reagents for repeated use at later dates. Reagents are labeled with expiration date.

Samples should be aliquoted and must be stored frozen at -20°C to avoid loss of bioactive human IFN-ω.

Reagents and materials not provided

Buffers and solutions can be prepared (see "Preparation of buffers and solutions" on page 1) or ordered at Thermo Fisher Scientific. We recommend the use of Thermo Fisher Scientific reagents to ensure a high quality assay performance.

Reagents and materials can be ordered as

- Support Pack Standard (BMS412), which includes buffers and solutions for 10 assays (listed at the table below, plates excluded);
- Support Pack Plus (BMS414), which includes buffers, solutions and plates for 10 assays (listed at the table below);
- separate items (for BMS Order Numbers see table below);

Reagents and Materials	BMS Order Numbers
Phosphate Buffered Saline (PBS)	BMS410.0010, 20x (10 mL)
Assay Buffer	BMS407.0050, 20x (50 mL)
Wash Buffer	BMS408.0500, 20x (500 mL)
Substrate Solution	BMS406.0100, ready to use (100 mL)
Stop Solution	BMS409.0100, ready to use (100 mL)
Microwell plate (We recommend F8 Immuno Modules Maxisorb, NUNC Order Number 468667.)	BMSPLATE

Precautions for use

All reagents should be considered as potentially hazardous. We therefore recommend that this product is handled only by those persons who have been trained in laboratory techniques and that it is used in accordance with the principles of good laboratory practice. Wear suitable protective clothing such as laboratory overalls, safety glasses and gloves. Care should be taken to avoid contact with skin or eyes. In the case of contact with skin or eyes wash immediately with water. See material safety data sheet(s) for specific advice.

Preparation of buffers and solutions

Note: The quality of BSA is a critical parameter for the test performance. We recommend Probumin™ Media Grade (e.g.: Millipore Order Number 81-068) or the use of the Thermo Fisher Scientific reagents (see "Reagents and materials not provided" on page 1).

Phosphate buffered saline (PBS)

NaCl	8.00 g
KCl	0.20 g
Na_2HPO_4 x 12 H_2O	2.85 g
KH_2PO_4	0.20 g
H_2O dest	adjust to 1 liter

Wash buffer

Add 0.5 mL Tween 20 to 1 liter of PBS and mix well.

Assay buffer

Bovine Serum Albumin (BSA)	5 g
Tween 20	0.5 mL
PBS	adjust to 1 liter

Fixing buffer

Sucrose	75 g
PBS	adjust to 500 mL

Substrate solution

1:2 mixture of H_2O_2 and Tetramethylbenzidine

Stop solution

1M Phosporic Acid (H_3PO_4)

Preparation of the microwell plate

Coating

1. Coating antibody final concentration is 1 µg/mL; 100 µL of the coating solution is added to each well. Dilute the coating antibody as following for one microtiter plate:

10.89 mL	PBS
0.11 mL	coating antibody (100 µg/mL)
11.00 mL	coating solution (1 µg/mL)

2. Immediately after coating, seal the plate with an adhesive film and store at 2-8°C over night, allowing the binding process to take place. Aspirate the contents of the wells and wash once with 400 µL of Wash Buffer according the washing procedure described in the test protocol below (see "Test protocol" on page 2).

Blocking and fixing

Blocking

Add 250 µL of Assay Buffer to each well and incubate at room temperature for 2 hours. Alternatively the plate may be blocked over night at 2-8°C. Blocked plates can be stored at 2-8°C for up to one week.

Fixing

To store the coated plates for longer than one week aspirate Assay Buffer and add 150 µL Fixing Buffer to each well. Incubate for 1 hour at room temperature. Aspirate Fixing Buffer and dry plates over night at 28°C. When sealed with desiccant, the plates can be stored at 2-8°C for 2 months.

Preparation of immunological reagents

Note: Centrifuge vials before opening to collect contents.

Preparation of standard:

1. The concentrated human IFN-ω standard must be diluted 1:100 with Assay Buffer just prior to use in a clean plastic test tube according to the following dilution scheme:

3 µL	conc. Standard Protein (60 ng/mL)
297 µL	Assay Buffer
300 µL	Diluted Standard Protein (600 pg/mL)

2. Shake gently to mix. After usage remaining diluted standard cannot be stored and has to be discarded.
3. Aliquot the concentrated standard and store at -20°C.

Preparation of Biotin-Conjugate:

Dilute conc. Biotin-Conjugate 1:1000 with Assay Buffer before use. Use within 30 min after preparation. For one microwell plate dilute the stock reagents as follows:

5.5 µL	conc. Biotin-Conjugate
5494.5 µL	Assay Buffer
5.5 mL	diluted Biotin-Conjugate

Preparation of Streptavidin-HRP:

Dilute conc. Streptavidin-HRP 1:7500 with Assay Buffer before use. Use within 30 min after preparation. For one microwell plate dilute the stock reagents according to the following dilution scheme:

Dilution I: 1:10 dilution of the conc. Streptavidin-HRP

2.0 µL	conc. Streptavidin-HRP
18.0 µL	Assay Buffer
20.0 µL	diluted Streptavidin-HRP

Dilution II: 1:750 dilution of the 1:10 prediluted Streptavidin-HRP

16.0 µL	prediluted Streptavidin-HRP
11984.0 µL	Assay Buffer
12.0 mL	diluted Streptavidin-HRP

Test protocol

1. Wash blocked or blocked and fixed plates twice with approximately 400 µL Wash Buffer per well, with thorough aspiration of microwell contents between washes. Allow the Wash Buffer to sit in the wells for about 10 – 15 seconds before aspiration. Take care not to scratch the surface of the microwells.

   After the last wash step, empty wells and tap microwell strips on absorbent pad or paper towel to remove excess Wash Buffer. Use the microwell plate immediately after washing. Alternatively microwell plate can be placed upside down on a wet absorbent paper for no longer than 15 minutes. Do not allow wells to dry.
2. Add 100 µL of Sample Diluent in duplicate to all standard wells. Pipette 100 µL of diluted standard (see "Preparation of standard: " on page 2), (concentration = 600.0 pg/mL) in duplicate into well A1 and A2. Mix the contents of wells A1 and A2 by repeated aspiration and ejection (concentration of standard 1, S1 = 300.0 pg/mL), and transfer 100 µL to wells B1 and B2, respectively (see Figure 1). Take care not to scratch the surface of the microwells. Continue this procedure 5 times, creating two serially diluted columns of human IFN-ω standard dilutions ranging from 300.0 to 4.7 pg/mL. Discard 100 µL from the last microwells (G1, G2). Final volume in all wells is 100 µL.

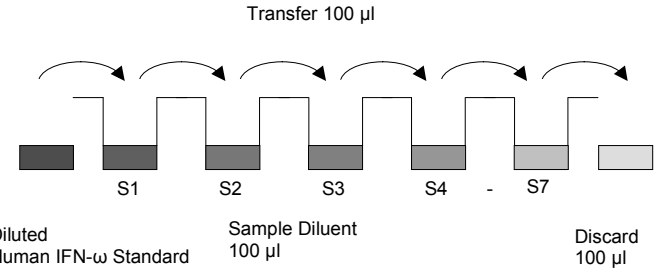

Fig. 1 Dilute standards - microwell plate

3. Add 100 µL of Sample Diluent in duplicate to blank wells.
4. Add 75 µL of Sample Diluent to the sample wells.
5. Add 25 µL of each sample in duplicate to the sample wells.
6. Prepare Biotin-Conjugate (see "Preparation of Biotin-Conjugate: " on page 2)
7. Add 50 µL of prepared Biotin-Conjugate to all wells.
8. Cover with an adhesive film and incubate at room temperature (18 to 25°C) for 2 hours, on a microplate shaker if available.
9. Prepare Streptavidin-HRP (see "Preparation of Streptavidin-HRP: " on page 2).
10. Remove adhesive film and empty wells. Wash microwells 3 times according to point 1. of the test protocol. Proceed immediately to the next step.
11. Add 100 µL of diluted Streptavidin-HRP to all wells, including the blank wells.
12. Cover with an adhesive film and incubate at room temperature (18° to 25°C) for 1 hour, on a microplate shaker if available.

13. Remove adhesive film and empty wells. Wash microwells 3 times according to point 1. of the test protocol. Proceed immediately to the next step.
14. Pipette 100 µL of Substrate Solution to all wells.
15. Incubate the microwell strips at room temperature (18° to 25°C) for about 10 min. Avoid direct exposure to intense light.

    Monitor the color development on the plate. The substrate reaction should be stopped (see next point of this protocol) before positive wells are no longer properly recordable. Determination of the ideal time period for color development has to be done individually for each assay.

    Add the stop solution when the highest standard has developed a dark blue color. Alternatively the color development can be monitored on a plate reader at 620 nm. The substrate reaction should be stopped as soon as Standard 1 has reached an OD of 0.9 – 0.95.
16. Stop the enzyme reaction by quickly pipetting 100 µL of Stop Solution into each well. It is important that the Stop Solution is spread quickly and uniformly throughout the microwells to completely inactivate the enzyme. Results must be read immediately after the Stop Solution is added, or within one hour if the microwell strips are stored at 2 - 8°C in the dark.
17. Read absorbance of each microwell on a spectro-photometer using 450 nm as primary wave length (you can use 620 nm as reference wave length; 610 nm to 650 nm is acceptable). Blank the plate reader according to the manufacturer's instructions by using the blank wells. Determine the absorbance of both the samples and the human IFN-ω standards.

Calculation of results

- Calculate the average absorbance values for each set of duplicate standards and samples. Duplicates should be within 20 percent of the mean value.
- Create a standard curve by plotting the mean absorbance for each standard concentration on the y-axis, against the human IFN-ω concentration on the x-axis. Draw a best fit curve through the points of the graph (a 5-parameter curve fit is recommended).
- To determine the concentration of soluble human IFN-ω for each sample, first calculate the mean absorbance value for the duplicate wells of the sample, then extend a horizontal line from this point on the y-axis to the standard curve. At the point of intersection, extend a vertical line to the x-axis and read the corresponding human IFN-ω concentration.
- If instructions in this protocol have been followed samples have been diluted 1:4 (25 µL sample + 75 µL Sample Diluent), the concentration read from the standard curve must be multiplied by the dilution factor (x 4).
- Calculation of samples with a concentration exceeding that of standard 1 may result in inaccurate, low human IFN-ω levels. Such samples require further external predilution according to expected human IFN-ω values with Sample Diluent in order to precisely quantitate the actual human IFN-ω level.
- Each testing facility should establish a control sample of known human IFN-ω concentration and run this additional control with each assay. If the values obtained are not within the expected range of this control, the assay results may be invalid.

A basic understanding of immunoassay development and technical experience in ELISA performance are conditional for the successful use of this matched antibody pair.

The protocol provided is just a guideline. The type of substrate as well as all other reagents not included in the matched antibody pair kit may influence the test characteristics.

Human IFN-ω module set characteristics

Specificity

The interference of circulating factors of the immune system was evaluated by spiking these proteins at physiologically relevant concentrations into human serum and cell culture supernatant. No cross-reactivity was detected, notably not with IFN-α and IFN-γ.

Expected values

A panel of serum samples from randomly selected apparently healthy donors (males and females) was tested for human IFN-ω. There were no detectable human IFN-ω levels found. Elevated human IFN-ω levels depend on the type of immunological disorder.

Additionally available components

Additional components of the Human IFN omega Matched Antibody Pair are available:

- Standard Protein (S): BMS233MSTS (for 10 assays) or BMS233S (for 1 assay)
- Coating Antibody (CA): BMS233MSTCA
- Sample Diluent (SD): BMS233MSTSD
- Biotin-Conjugate (BK): BMS233MSTBK
- Streptavidin-HRP (PK): BMS233MSTPK

Contact your sales representative.

Customer and technical support

Visit **thermofisher.com/support** for the latest in services and support, including:

- Worldwide contact telephone numbers
- Product support, including:
  - Product FAQs
  - Software, patches, and updates
  - Training for many applications and instruments
- Order and web support
- Product documentation, including:
  - User guides, manuals, and protocols
  - Certificates of Analysis
  - Safety Data Sheets (SDSs; also known as MSDSs)

    Note: For SDSs for reagents and chemicals from other manufacturers, contact the manufacturer.

Limited product warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale found on Life Technologies' website at **www.thermofisher.com/us/en/home/global/terms-and-conditions.html**. If you have any questions, please contact Life Technologies at **www.thermofisher.com/support**.

Manufacturer's address: Bender MedSystems GmbH | Campus Vienna Biocenter 2 | 1030 Vienna, Austria