BioVendor | ® Group

ENG

Product Data Sheet:

HUMAN INTERLEUKIN-12/INTERLEUKIN-23 P40 ELISA

Catalogue number:

RAF034R

For research use only!

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1.   INTENDED USE

The Human IL12/IL23 p40 ELISA Kit is an enzyme-linked immunosorbent assay for the quantitative detection of human IL12/IL23 (p40).
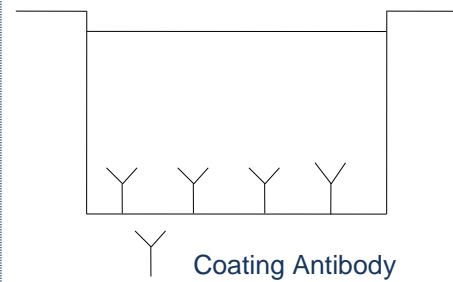
2.   SUMMARY

IL23 and IL12 are heterodimeric cytokines that share the p40 subunit, but have unique second subunits, IL23 (p19) and IL12 (p35).

IL12 (p70) is a heterodimer composed of a 35kDa subunit (IL12 [p35] and the 40kDa subunit (IL12 [p40]) that binds the IL12 receptor. The p40 subunit is secreted in excess of IL12 (p35) and can also associate with p19 (IL-23 [P19] or IL23A) to form IL23. The p19 subunit shares sequence similarity with IL6 subfamily members and is distantly related to the p35 subunit of IL12. It shows no biological activity by itself and combines with the p40 subunit of IL12 to form a biologically active, composite cytokine, IL23. IL23 signals through the IL23 receptor and has functions that overlap with and are distinct from those of IL12. Unlike IL12, IL23 has no effect on naive T-cells but selectively activates memory T-cells to secrete IFNγ and IL17. IL23 is more potent than IL12 in inducing long-lasting T-cell responses to viral antigens and can induce tumor-directed cytotoxic T-cell responses.
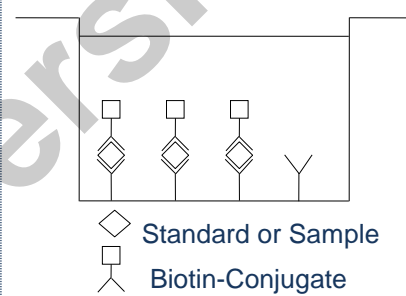
3. PRINCIPLES OF THE TEST

An anti-human IL-12/IL-23 p40 coating antibody is adsorbed onto microwells.	Figure 1 **Coated Microwell** Coating Antibody
Human IL-12/IL-23 p40 present in the sample or standard binds to antibodies adsorbed to the microwells. A biotin-conjugated anti-human IL-12/IL-23 p40 antibody is added and binds to human IL-12/IL-23 p40 captured by the first antibody.	Figure 2 **First Incubation** Standard or Sample Biotin-Conjugate
Following incubation unbound biotin-conjugated anti-human IL-12/IL-23 p40 antibody is removed during a wash step. Streptavidin-HRP is added and binds to the biotin-conjugated anti-human IL-12/IL-23 p40 antibody.	Figure 3 **Second Incubation** Streptavidin-HRP
Following incubation unbound Streptavidin-HRP is removed during a wash step, and substrate solution reactive with HRP is added to the wells.	Figure 4 **Third Incubation** Substrate
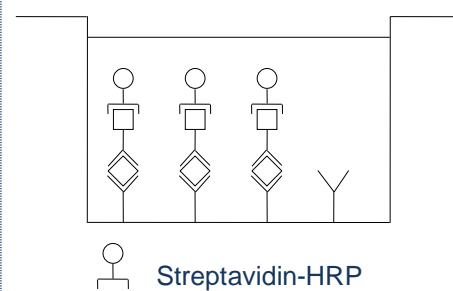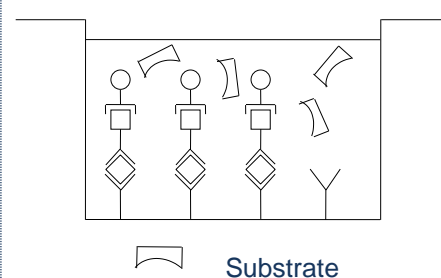
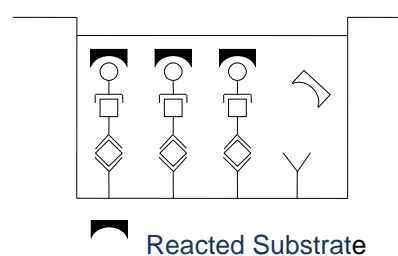| A coloured product is formed in proportion to the amount of human IL-12/IL-23 p40 present in the sample or standard. The reaction is terminated by addition of acid and absorbance is measured at 450 nm. A standard curve is prepared from 7 human IL-12/IL-23 p40 standard dilutions and human IL-12/IL-23 p40 sample concentration determined. | Figure 5 Reacted Substrate |

4. REAGENTS PROVIDED

1 aluminum pouch with a Microwell Plate (12 strips of 8 wells each) coated with polyclonal antibody to human IL12/IL23 (p40)

1 vial (70 µL) Biotin-Conjugate anti-human IL12/IL23 (p40) monoclonal antibody

1 vial (150 µL) Streptavidin-HRP

2 vials human IL12/IL23 (p40) Standard lyophilized, 4 ng/mL uponreconstitution

1 vial (12 mL) Sample Diluent

1 vial (5 mL) Assay Buffer Concentrate 20x (PBS with 1% Tween™ 20, 10% BSA)

1 bottle (50 mL) Wash Buffer Concentrate 20x (PBS with 1% Tween™ 20)

1 vial (15 mL) Substrate Solution (tetramethyl-benzidine)

1 vial (15 mL) Stop Solution (1M Phosphoric acid)

4 Adhesive Films

5. STORAGE INSTRUCTIONS – ELISA KIT

Store kit reagents between 2°C to 8°C. Immediately after use remaining reagents should be returned to cold storage (2°C to 8°C). Expiry of the kit and reagents is stated on labels.

Expiry of the kit components can be guaranteed only if the components are stored properly, and if, in case of repeated use of one component, this reagent is not contaminated by the first handling.

6. SPECIMEN COLLECTION AND STORAGE INSTRUCTIONS

Cell culture supernatant, serum and plasma (citrate, heparin) were tested with this assay. Other biological samples might be suitable for use in the assay. Remove serum or plasma from the clot or cells as soon as possible after clotting and separation. Pay attention to a possible *Hook Effect* due to high sample concentrations (see "Calculation of results" chap.11).

Samples containing a visible precipitate must be clarified prior to use in the assay. Do not use grossly hemolyzed or lipemic specimens. Samples should be aliquoted and must be stored frozen at –20°C to avoid loss of bioactive human IL12/IL23 (p40). If samples are to be run within 24 hours, they may be stored at 2°C to 8°C (for sample stability refer to "Sample stability"13.5). Avoid repeated freeze-thaw cycles. Prior to assay, the frozen sample should be brought to room temperature slowly and mixed gently.

7. MATERIALS REQUIRED BUT NOT PROVIDED

- 5 mL and 10 mL graduated pipettes
- 5 µL to 1000 µL adjustable single channel micropipettes with disposable tips
- 50 µL to 300 µL adjustable multichannel micropipette with disposable tips
- Multichannel micropipette reservoir
- Beakers, flasks, and cylinders necessary for preparation of reagents
- Device for delivery of wash solution (multichannel wash bottle or automatic wash system)
- Microwell strip reader capable of reading at 450 nm (620 nm asoptional reference wave length)
- Glass-distilled or deionized water
- Statistical calculator with program to perform regression analysis

8. PRECAUTIONS FOR USE

– All chemicals 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) and/or safety statement(s) for specific advice.

– Reagents are intended for research use only and are not for use in diagnostic or therapeutic procedures.

– Do not mix or substitute reagents with those from other lots or other sources.

– Do not use kit reagents beyond expiration date on label.

– Do not expose kit reagents to strong light during storage or incubation.

– Do not pipet by mouth.

– Do not eat or smoke in areas where kit reagents or samples are handled.

– Avoid contact of skin or mucous membranes with kit reagents or samples.

– Rubber or disposable latex gloves should be worn while handling kit reagents or samples.

– Avoid contact of substrate solution with oxidizing agents and metal.

– Avoid splashing or generation of aerosols.

– To avoid microbial contamination or cross-contamination of reagents or samples that may invalidate the test, use disposable pipette tips and/or pipettes.

– Use clean, dedicated reagent trays for dispensing the conjugate and substrate reagent.

– Exposure to acid inactivates the conjugate.

– Glass-distilled water or deionized water must be used for reagent preparation.

– Substrate solution must be at room temperature prior to use.

– Decontaminate and dispose samples and all potentially contaminated materials as if they could contain infectious agents. The preferred method of decontamination is autoclaving for a minimum of 1 hour at 121.5°C.

– Liquid wastes not containing acid and neutralized waste may be mixed with sodium hypochlorite in volumes such that the final mixture contains 1.0% sodium hypochlorite. Allow 30 minutes for effective decontamination. Liquid waste containing acid must be neutralized prior to the addition of sodium hypochlorite.

9. PREPARATION OF REAGENTS

Buffer concentrates should be brought to room temperature and should be diluted before starting the test procedure.

If crystals have formed in **buffer concentrates**, warm them gently until crystals have completely dissolved.

9.1 Wash buffer (1x)

- Pour entire contents (50 mL) of the **Wash Buffer Concentrate (20x)** into a clean 1000 mL graduated cylinder. Bring to final volume of 1000 mL with glass-distilled or deionized water.
- Mix gently to avoid foaming.
- Transfer to a clean wash bottle and store at 2°C to 25°C. Please note that Wash Buffer (1x) is stable for 30 days.
- Wash Buffer (1x) may also be prepared as needed according to the following table:

Number of Strips	Wash Buffer Concentrate (20x) (mL)	Distilled Water (mL)
1–6	25	475
1–12	50	950

9.2 Assay buffer (1x)

Pour the entire contents (5 mL) of the **Assay Buffer Concentrate (20x)** into a clean 100 mL graduated cylinder. Bring to final volume of 100 mL with distilled water. Mix gently to avoid foaming.

Store at 2°C to 8°C. Please note that the Assay Buffer (1x) is stable for 30 days.

Assay Buffer (1x) may also be prepared as needed according to the following table:

Number of Strips	Assay Buffer Concentrate (20x) (mL)	Distilled Water (mL)
1–6	2.5	47.5
1–12	5.0	95.0

9.3 Biotin-Conjugate

Note: Biotin-Conjugate should be used within 30 minutes after dilution.

Make a 1:100 dilution of the concentrated Biotin-Conjugate solution with Assay Buffer (1x) in a clean plastic tube as needed according to the following table:

Number of Strips	Biotin-Conjugate (mL)	Assay Buffer (1x) (mL)
1–6	0.03	2.97
1–12	0.06	5.94

9.4 Streptavidin-HRP

Note: The Streptavidin-HRP should be used within 30 minutes after dilution.

Make a 1:250 dilution of the concentrated Streptavidin-HRP solution with Assay Buffer (1x) in a clean plastic tube as needed according to the following table:

Number of Strips	Streptavidin-HRP (mL)	Assay Buffer (1x) (mL)
1–6	0.024	5.976
1–12	0.048	11.952

9.5 Human IL12/IL23 (p40) standard

Reconstitute human IL12/IL23 (p40) standard by addition of distilled water. Reconstitution volume is stated on the Quality Control Sheet. Swirl or mix gently to insure complete and homogeneous solubilization (concentration of reconstituted standard = 4 ng/mL).
Allow the standard to reconstitute for 10–30 minutes. Mix well prior to making dilutions.
The standard has to be used immediately after reconstitution and cannot be stored.

9.5.1 External standard dilution

Label 7 tubes, one for each standard point: S1, S2, S3, S4, S5, S6, S7. 2.

Prepare 1:2 serial dilutions for the standard curve as follows:

Pipette 225 µL of Sample Diluent into each tube.

Pipette 225 µL of reconstituted standard (concentration of standard = 4 ng/mL) into the first tube, labeled S1, and mix (concentration of standard 1 = 2 ng/mL).

Pipette 225 µL of this dilution into the second tube, labeled S2, and mix thoroughly before the next transfer.

Repeat serial dilutions 5 more times thus creating the points of the standard curve (see Figure 6). Sample Diluent serves as blank.

Fig. 6 Dilute standards - tubes.

Transfer 225 µl

S1   S2   S3   S4   -   S7

Reconstituted

Human IL12/IL-23 p40

standard

Sample Diluent

225 µl

Discard

225 µl
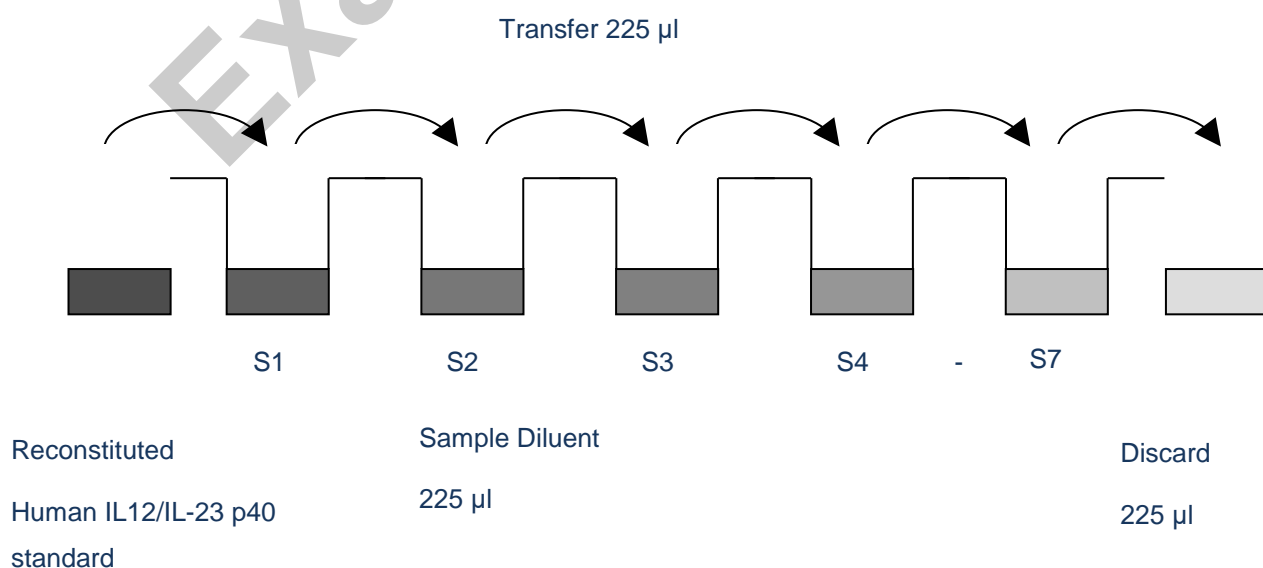
10. TEST PROTOCOL

<u>Note</u>: In case of incubation without shaking the obtained O.D. values may be lower than indicated below. Nevertheless the results are still valid.

1. Determine the number of microwell strips required to test the desired number of samples plus appropriate number of wells needed for running blanks and standards. Each sample, standard, blank and optional control sample should be assayed in duplicate. Remove extra microwell strips from holder and store in foil bag with the desiccant provided at 2°C to 8°C sealed tightly.

2. Wash the microwell strips 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 strips immediately after washing. Alternatively microwell strips can be placed upside down on a wet absorbent paper for not longer than 15 minutes. Do not allow wells to dry.

3. Standard dilution on the microwell plate (alternatively the standard dilution can be prepared in tubes, see "External standard dilution" 9.5.1.):

    Add 100 µL of Sample Diluent in duplicate to all standard wells. Pipette 100 µL of prepared standard (see "External standard dilution", concentration = 4000.0 pg/mL) in duplicate into well A1 and A2 (see Table 1). Mix the contents of wells A1 and A2 by repeated aspiration and ejection (concentration of standard 1, S1 = 2000.0 pg/mL), and transfer 100 µL to wells B1 and B2, respectively (see Figure 7). Take care not to scratch the inner surface of the microwells. Continue this procedure 5 times, creating two rows of human IL12/IL23 (p40) standard dilutions ranging from 2000 to 31 pg/mL. Discard 100 µL of the contents from the last microwells (G1/G2) used.

Fig. 7 Dilute standards - microwell plate.

Transfer 100 µl

S1   S2   S3   S4   -   S7

Reconstituted

Human IL-12/IL-23 p40

standard

Sample Diluent

100 µl

Discard

100 µl
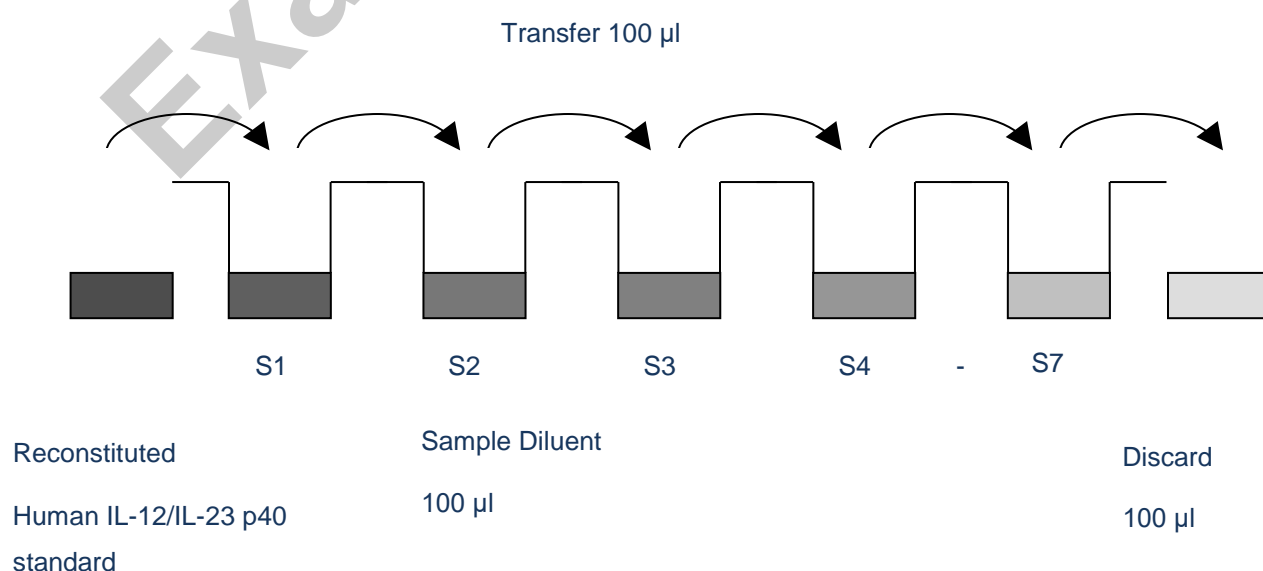
In case of an external standard dilution (see "External standard dilution" 9.5.1), pipette 100 µL of these standard dilutions (S1–S7) in the standard wells according to Table 1.

Table 1 Example of the arrangement of blanks, standards, and samples in the microwell strips.

	1	2	3	4
A	Standard 1 2000.0 pg/mL	Standard 1 2000.0 pg/mL	Sample 1	Sample 1
B	Standard 2 1000.0 pg/mL	Standard 2 1000.0 pg/mL	Sample 2	Sample 2
C	Standard 3 500.0 pg/mL	Standard 3 500.0 pg/mL	Sample 3	Sample 3
D	Standard 4 250.0 pg/mL	Standard 4 250.0 pg/mL	Sample 4	Sample 4
E	Standard 5 125.0 pg/mL	Standard 5 125.0 pg/mL	Sample 5	Sample 5
F	Standard 6 62.5 pg/mL	Standard 6 6.25 pg/mL	Sample 6	Sample 6
G	Standard 7 31.3 pg/mL	Standard 7 31.3 pg/mL	Sample 7	Sample 7
H	Blank	Blank	Sample 8	Sample 8

4. Add 100 µL of Sample Diluent in duplicate to the blank wells.
5. Add 50 µL of Sample Diluent to the sample wells.
6. Add 50 µL of each sample in duplicate to the sample wells.
7. Prepare Biotin-Conjugate (see "Biotin-Conjugate" 9.3).
8. Add 50 µL of Biotin-Conjugate to all wells.
9. Cover with an adhesive film and incubate at room temperature (18°C to 25°C) for 3 hours on a microplate shaker.
10. Prepare Streptavidin-HRP (see "Streptavidin-HRP" 9.4).
11. Remove adhesive film and empty wells. Wash microwell strips 6 times according to step 2. Proceed immediately to the next step. 12. Add 100 µL of diluted Streptavidin-HRP to all wells, including the blank wells.
13. Cover with an adhesive film and incubate at room temperature (18°C to 25°C) for 1 hour on a microplate shaker.
14. Remove adhesive film and empty wells. Wash microwell strips 6 times according to step 2. Proceed immediately to the next step.
15. Pipet 100 µL of TMB Substrate Solution to all wells.
16. Incubate the microwell strips at room temperature (18°C to 25°C) for about 10 minutes. Avoid direct exposure to intense light.

    The color development on the plate should be monitored and the substrate reaction stopped (see next step) 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.

    It is recommended to add the stop solution when the highest standard has developed a dark blue color. Alternatively the color development can be monitored by the ELISA reader at

620 nm. The substrate reaction should be stopped as soon as standard 1 has reached an OD of 0.9–0.95.

17. 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°C to 8°C in the dark.

18. Read absorbance of each microwell on a spectrophotometer using 450 nm as the primary wave length (optionally 620 nm as the 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 standards.

11. CALCULATION OF RESULTS

1. Calculate the average absorbance values for each set of duplicate standards and samples. Duplicates should be within 20% of the mean value.

2. Create a standard curve by plotting the mean absorbance for each standard concentration on the ordinate against the human IL-12/IL23 (p40) concentration on the abscissa. Draw a best fit curve through the points of the graph (a 5-parameter curve fit is recommended).

3. To determine the concentration of circulating human IL12/IL23 (p40) for each sample, first find the mean absorbance value on the ordinate and extend a horizontal line to the standard curve. At the point of intersection, extend a vertical line to the abscissa and read the corresponding human IL12/IL23 (p40) concentration.

4. If instructions in this protocol have been followed, samples have been diluted 1:2 (50 µL sample + 50 µL Sample Diluent) and the concentration read from the standard curve must be multiplied by the dilution factor (x 2).

5. Calculation of samples with a concentration exceeding standard 1 will result in incorrect, low human IL12/IL23 (p40) levels (Hook Effect). Such samples require further external predilution according to expected human IL12/IL23 (p40) values with Sample Diluent in order to precisely quantitate the actual human IL12/IL23 (p40) level.

6. It is suggested that each testing facility establishes a control sample of known human IL12/IL23 (p40) concentration and runs this additional control with each assay. If the values obtained are not within the expected range of the control, the assay results may be invalid.

7. A representative standard curve is shown in Figure 8. Note: Do not use this standard curve to derive test results. Each laboratory must prepare a standard curve for each group of microwell strips assayed.
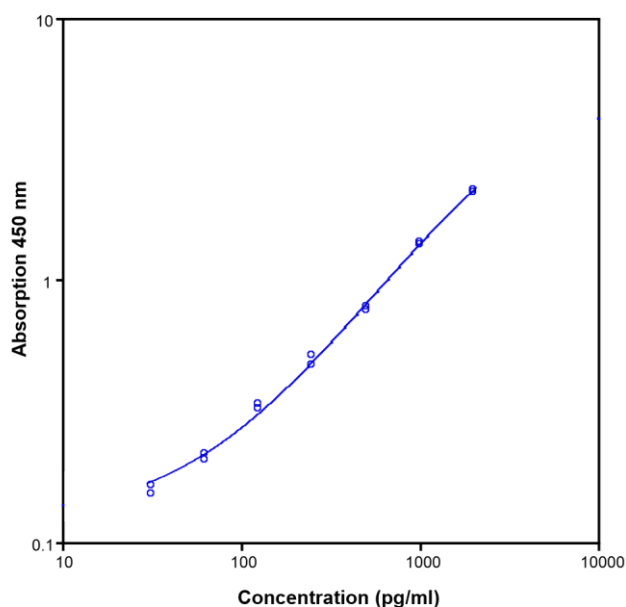
Fig. 8 Representative standard curve for Human IL12/IL23 p40 ELISA Kit. Human IL12/IL23 (p40) was diluted in serial 2-fold steps in Sample Diluent.

Table 2 Typical data using the Human IL12/IL23 p40 ELISA Kit.

Measuring wavelength: 450 nm

Reference wavelength: 620 nm

Standard	Human IL12/IL23 (p40) concentration (pg/mL)	O.D. at 450 nm	Mean O.D. at 450 nm	C.V. (%)
1	2000.0	2.177 2.222	2.199	1.0
2	1000.0	1.377 1.398	1.388	0.7
3	500.0	0.771 0.794	0.783	1.5
4	250.0	0.476 0.521	0.499	4.5
5	125.0	0.324 0.337	0.330	2.0
6	62.5	0.207 0.218	0.212	2.6
7	31.3	0.154 0.166	0.160	3.8
Blank	0	0.088 0.096	0.092	4.3

The OD values of the standard curve may vary according to the conditions of assay performance (e.g., operator, pipetting technique, washing technique, or temperature effects).

Furthermore, shelf life of the kit may affect enzymatic activity and thus color intensity. Values measured are still valid.

12. LIMITATIONS

- Because exact conditions may vary from assay to assay, a standardcurve must be established for every run.
- Bacterial or fungal contamination of either screen samples orreagents or cross-contamination between reagents may cause erroneous results.
- Disposable pipette tips, flasks, or glassware are preferred, reusable glassware must be washed and thoroughly rinsed of all detergents before use.
- Improper or insufficient washing at any stage of the procedure will result in either false positive or false negative results. Empty wells completely before dispensing fresh wash solution, fill with Wash Buffer as indicated for each wash cycle and do not allow wells to sit uncovered or dry for extended periods.
- The use of radioimmunotherapy has significantly increased the number of patients with human anti-mouse IgG antibodies (HAMA). HAMA may interfere with assays utilizing murine monoclonal antibodies leading to both false positive and false negative results. Serum samples containing antibodies to murine immunoglobulins can still be analyzed in such assays when murine immunoglobulins (serum, ascitic fluid, or monoclonal antibodies of irrelevant specificity) are added to the sample.

13. PERFORMANCE CHARACTERISTICS

13.1   Sensitivity

The limit of detection of human IL12/IL23 (p40) defined as the analyte concentration resulting in an absorbance significantly higher than that of the dilution medium (mean plus 2 standard deviations) was determined to be <10 pg/mL (mean of 6 independent assays).

13.2 Reproducibility

13.2.1 Intra-assay

Reproducibility within the assay was evaluated in 3 independent experiments. Each assay was carried out with 6 replicates of 8 serum samples containing different concentrations of human IL12/IL23 (p40). Two standard curves were run on each plate. Data below show the mean human IL12/IL23 (p40) concentration and the coefficient of variation for each sample (see Table 3). The calculated overall intra-assay coefficient of variation was 4.7%.

Table 3 The mean human IL12/IL23 (p40) concentration and the coefficient of variation for each sample.

Sample	Experiment	Mean human IL12/IL23 (p40) concentration (pg/mL)	Coefficient of variation (%)
1	1	217	7.4
	2	188	4.9
	3	167	9.6
2	1	359	3.6
	2	356	4.7
	3	362	5.2
3	1	102	5.9
	2	111	3.6
	3	120	5.7
4	1	78	7.0
	2	100	6.5
	3	70	7.3
5	1	3354	3.5
	2	3492	5.7
	3	3203	6.6
6	1	2227	1.9
	2	2196	2.2
	3	2162	2.0
7	1	1601	1.7
	2	1617	2.1
	3	1527	1.6
8	1	715	2.7
	2	696	7.2
	3	701	4.5

13.2.2 Inter-assay

Assay-to-assay reproducibility within one laboratory was evaluated in 3 independent experiments. Each assay was carried out with 6 replicates of 8 serum samples containing different concentrations of human IL12/IL23 (p40). Two standard curves were run on each plate. Data below show the mean human IL12/IL23 (p40) concentration and the coefficient of variation calculated on 18 determinations of each sample (see Table 4). The calculated overall inter-assay coefficient of variation was 6.4%.

Table 4 The mean human IL12/IL23 (p40) concentration and the coefficient of variation of each sample.

Sample	Mean human IL12/IL23 (p40) concentration (pg/mL)	Coefficient of variation (%)
1	190	13.2
2	359	0.7
3	111	8.0
4	83	19.1
5	3350	4.3
6	2195	1.5
7	1582	3.1
8	704	1.4

13.3 Spike recovery

The spike recovery was evaluated by spiking 4 levels of human IL12/IL23 (p40) into serum. Recoveries were determined in 3 independent experiments with 8 serum samples. The amount of endogenous human IL12/IL23 (p40) in unspiked serum was subtracted from the spike values. The recovery ranged from 65–75% with an overall mean recovery of 71%.

13.4 Dilution parallelism

Serum samples with different levels of human IL12/IL23 (p40) were analyzed at serial 2-fold dilutions with 4 replicates each. The recovery ranged from 101.4–120.2% with an overall recovery of 109.8%.

Sample	Dilution	Human IL12/IL23 (p40) (pg/mL)		Recovery of expected concentration (%)
		Expected concentration	Observed concentration	
1	1:2	–	2346	–
	1:4	1173	1256	107.1
	1:8	628	707	112.6
	1:16	354	381	107.9
2	1:2	–	2160	–
	1:4	1080	1257	116.4
	1:8	628	723	115.1
	1:16	362	405	112.0
3	1:2	–	3069	–
	1:4	1535	1622	105.7
	1:8	811	822	101.4
	1:16	411	418	101.6
4	1:2	–	1857	–
	1:4	929	990	106.6
	1:8	495	548	110.7
	1:16	274	329	120.2

13.5. Sample stability

13.5.1 Freeze-thaw stability

Aliquots of serum plasma cellculture supernatant samples (spiked or unspiked) were stored at –20°C and thawed 5 times, and the human IL12/IL23 (p40) levels determined. There was no significant loss of human IL12/IL23 (p40) immunoreactivity detected by freezing and thawing.

13.5.2 Storage stability

Aliquots of serum samples (spiked or unspiked) were stored at –20°C,

2°C to 8°C, room temperature, and at 37°C, and the human IL12/IL23 (p40) level determined after 24 hours. There was no significant loss of human IL12/IL23 (p40) immunoreactivity detected during storage under above conditions.

13.6 Specificity

The assay detects both natural and recombinant human IL12/IL23 (p40). The interference of circulating factors of the immune system was evaluated by spiking these proteins at physiologically relevant concentrations. There was no cross-reactivity observed with IL-1β, IL2, IL-4, IL-5, IL-6, IL-8, IL-10, IFNγ, TNFα, and TNFβ. BMS2013 also detects IL23 and IL12p70.

13.7 Expected values

A panel of sera samples from randomly selected apparently healthy donors (males and females) was tested for human IL12/IL23 (p40). The detected human IL12/IL23 (p40) levels ranged between 0 and 521 pg/mL with a mean level of 138 pg/mL and a standard deviation of 127 pg/mL for sera samples, between 0 and 825 pg/mL with a mean level of 190 pg/mL and a standard deviation of 196 pg/mL for plasma (citrate) samples and between 0 and 266 pg/mL with a mean level of 67 pg/mL and a standard deviation of 73 pg/mL for plasma (heparin) samples. Elevated human IL12/IL23 (p40) levels depend on the type of immunological disorder.

Panels of 40 serum as well as citrate and heparin plasma samples from randomly selected apparently healthy donors (males and females) was tested for human IL12/IL23 (p40). The levels measured may vary with the sample collection used.

Table 5 Detected human IL12/IL23 (p40) levels.

Sample matrix	Number of samples evaluated	Range (pg/mL)	Detectable (%)	Mean of detectable (pg/mL)
Serum	40	nd [1]–520.8	72.5	184.0
Plasma (citrate)	40	nd–824.9	67.5	267.4
Plasma (heparin)	40	nd–266.2	47.5	123.8

14. REAGENT PREPARATION SUMMARY

14.1 Wash buffer (1x)

Add Wash Buffer Concentrate 20x (50 mL) to 950 mL distilled water.

Number of Strips	Wash Buffer Concentrate (mL)	Distilled Water (mL)
1–6	25	475
1–12	50	950

14.2 Assay buffer (1x)

Add Assay Buffer Concentrate 20x (5 mL) to 95 mL distilled water.

Number of Strips	Assay Buffer Concentrate (mL)	Distilled Water (mL)
1–6	2.5	47.5
1–12	5.0	95.0

14.3 Biotin-Conjugate

Make a 1:100 dilution of Biotin-Conjugate in Assay Buffer (1x):

Number of Strips	Biotin-Conjugate (mL)	Assay Buffer (1x) (mL)
1–6	0.03	2.97
1–12	0.06	5.94

14.4 Streptavidin-HRP

Make a 1:250 dilution of Streptavidin-HRP in Assay Buffer (1x):

Number of Strips	Streptavidin-HRP (mL)	Assay Buffer (1x) (mL)
1–6	0.024	5.976
1–12	0.048	11.952

14.5 Human IL12/IL23 (p40) standard

Reconstitute lyophilized human IL12/IL23 (p40) standard with distilled water. (Reconstitution volume is stated on the Quality Control Sheet.)

15. TEST PROTOCOL SUMMARY

Note: If instructions in this protocol have been followed, samples have been diluted 1:2 (50 µL sample + 50 µL Sample Diluent) and the concentration read from the standard curve must be multiplied by the dilution factor (x 2).

1. Determine the number of microwell strips required.
2. Wash microwell strips twice with Wash Buffer.
3. Standard dilution on the microwell plate: Add 100 µL Sample Diluent, in duplicate, to all standard wells. Pipette 100 µL prepared standard into the first wells and create standard dilutions by transferring 100 µL from well to well. Discard 100 µL from the last wells.
4. Alternatively external standard dilution in tubes (see "External standard dilution" 9.5.1): Pipet 100 µL of these standard dilutions in the microwell strips.
5. Add 100 µL Sample Diluent, in duplicate, to the blank wells.
6. Add 50 µL Sample Diluent to sample wells.
7. Add 50 µL sample in duplicate, to designated sample wells.
8. Prepare Biotin-Conjugate.
9. Add 50 µL Biotin-Conjugate to all wells.
10. Cover microwell strips and incubate 3 hours at room temperature (18°C to 25°C).
11. Prepare Streptavidin-HRP.
12. Empty and wash microwell strips 6 times with Wash Buffer.
13. Add 100 µL diluted Streptavidin-HRP to all wells.
14. Cover microwell strips and incubate 1 hour at room temperature (18°C to 25°C).
15. Add 100 µL of TMB Substrate Solution to all wells.
16. Incubate the microwell strips for about 10 minutes at room temperature (18°C to 25°C).
17. Add 100 µL Stop Solution to all wells.
18. Blank microwell reader and measure color intensity at 450 nm.

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