ELISA PRODUCT INFORMATION & MANUAL

Human IL-9 ELISA Kit (Colorimetric)

NBP1-84825

Enzyme-linked Immunosorbent Assay for quantitative detection. For research use only.
Not for diagnostic or therapeutic procedures.

www.novusbio.com - P: 303.730.1950 - P: 888.506.6887 - F: 303.730.1966 - technical@novusbio.com

Human IL-9 ELISA Kit

Enzyme-linked Immunosorbent Assay for quantitative detection of human IL-9

Catalog Numbers NBP1-84825

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 Technical Support.

Product description

The Human IL-9 ELISA Kit is an enzyme-linked immunosorbent assay for the quantitative detection of human IL-9.

Interleukin-9 (IL-9) is a proinflammatory cytokine historically believed to be involved in type 2 immune responses. However, recent evidence suggests IL-9 may be secreted by other T Helper lineages such as Treg and Th17 in addition to a new category called Th9. This Th9 lineage can either be derived from Th2 cells with TGF β or differentiated directly from naïve CD4+ T cells with TGF β and IL-4. The IL-9 expression in subsets such as Treg and Th17 illustrates the plasticity of cells to reprogram to alternative fates.

IL-9 is a member of the common cytokine receptor γ chain-dependent family of cytokines which also includes IL-2, IL-4, IL-7, IL-15 and IL-21. IL-9 is an extensively glycosylated protein of 14 kDa containing ten cysteine residues involved in disulfide bonding. The human gene maps to chromosome 5q31-32 which is a chromosomal region sometimes deleted in patients with myelodysplastic syndrome. Its pleiotropic effects on Th2 lymphocytes, B lymphocytes, mast cells, eosinophils, IgE production and gut and airway epithelial cells have implicated IL-9 in asthma and other allergy-related disorders. The existence of an IL-9-mediated autocrine loop has been suggested for some malignancies such as Hodgkin's disease and large cell anaplastic lymphoma for Hodgkin's cell lines. IL-9 is expressed by Reed-Sternberg cells and Hodgkin lymphoma cells and some large aplastic lymphoma cells, while non-Hodgkin lymphomas and peripheral T-cell lymphomas do not express it.

Principles of the test

An anti-human IL-9 coating antibody is adsorbed onto microwells.

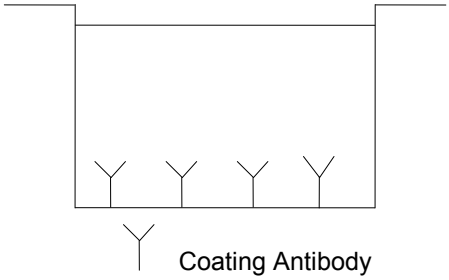

Fig. 1 Coated microwell

Human IL-9 present in the sample or standard binds to antibodies adsorbed to the microwells and a biotin-conjugated anti-human IL-9 antibody is added and binds to human IL-9 captured by the first antibody.

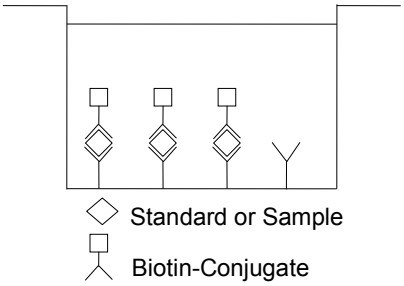

Fig. 2 First incubation

Following incubation unbound biotin-conjugated anti-human IL-9 antibody is removed during a wash step. Streptavidin-HRP is added and binds to the biotin-conjugated anti-human IL-9 antibody.

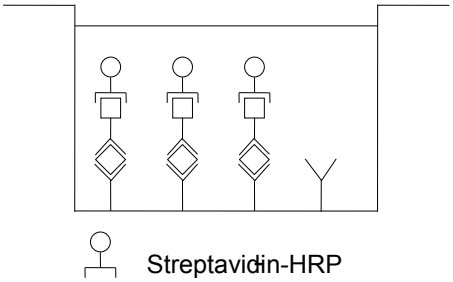

Fig. 3 Second incubation

Following incubation unbound Streptavidin-HRP is removed during a wash step, and substrate solution reactive with HRP is added to the wells.

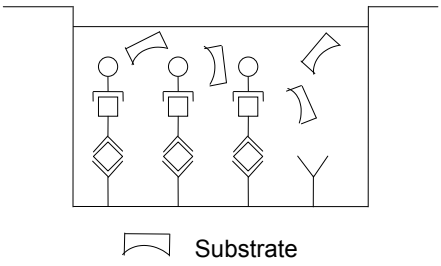

Fig. 4 Third incubation

A colored product is formed in proportion to the amount of human IL-9 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-9 standard dilutions and human IL-9 sample concentration determined.

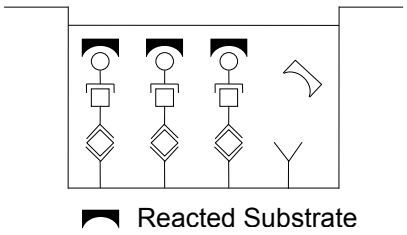

Fig. 5 Fourth incubation

Reagents provided

Reagents for human IL-9 ELISA NBP1-84825 (96 tests)

1 aluminum pouch with a Microwell Plate coated with monoclonal antibody to human IL-9

1 vial (70 µL) Biotin-Conjugate anti-human IL-9 monoclonal antibody

1 vial (150 µL) Streptavidin-HRP

2 vials human IL-9 Standard lyophilized, 200 pg/mL upon reconstitution

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 bottle (12 mL) Sample Diluent

1 bottle (5 mL) Calibrator Diluent

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

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

4 Adhesive Films

Reagents for human IL-9 ELISA NBP1-84825 (10x96 tests)

10 aluminum pouch with a Microwell Plate coated with monoclonal antibody to human IL-9

10 vials (70 µL) Biotin-Conjugate anti-human IL-9 monoclonal antibody

4 vials (150 µL) Streptavidin-HRP

10 vials human IL-9 Standard lyophilized, 200 pg/mL upon reconstitution

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

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

6 bottles (12 mL) Sample Diluent

5 bottles (5 mL) Calibrator Diluent

10 vials (15 mL) Substrate Solution (tetramethyl-benzidine)

1 vial (100 mL) Stop Solution (1M Phosphoric acid)

20 Adhesive Films

Storage instructions – ELISA kit

Store kit reagents between 2°C and 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 only be guaranteed 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.

Sample collection and storage instructions

Cell culture supernatant, serum, and plasma (EDTA, 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.

Samples containing a visible precipitate must be clarified prior to use in the assay. Do not use grossly hemolyzed or lipemic samples.

Samples should be aliquoted and must be stored frozen at –20°C to avoid loss of bioactive human IL-9. If samples are to be run within 24 hours, they may be stored at 2–8°C (for stability refer to "Sample stability" on page 6). Avoid repeated freeze-thaw cycles. Prior to assay, the frozen sample should be brought to room temperature slowly and mixed gently.

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, cylinders necessary for preparation of reagents
- Device for delivery of wash solution (multichannel wash bottle or automatic wash system)
- Microplate shaker
- Microwell strip reader capable of reading at 450 nm (620 nm as optional reference wave length)
- Glass-distilled or deionized water
- Statistical calculator with program to perform regression analysis

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) 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.

Preparation of reagents

1. Buffer Concentrates should be brought to room temperature and should be diluted before starting the test procedure.
2. If crystals have formed in the Buffer Concentrates, warm them gently until they have completely dissolved.

Wash buffer (1x)

1. 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.
2. Transfer to a clean wash bottle and store at 2° to 25°C. Please note that Wash Buffer (1x) is stable for 30 days.
3. 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

Assay buffer (1x)

1. 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.
2. Store at 2° to 8°C. Please note that the Assay Buffer (1x) is stable for 30 days.
3. 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

Biotin-Conjugate

Note: The 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

Streptavidin-HRP

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

Make a 1:200 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	0.03	5.97
1 - 12	0.06	11.94

Human IL-9 standard

1. Reconstitute human IL-9 standard by addition of Calibrator Diluent. Reconstitution volume is stated on the label of the standard vial. Swirl or mix gently to insure complete and homogeneous solubilization (concentration of reconstituted standard = 200 pg/mL).
2. Allow the standard to reconstitute for 10-30 minutes. Mix well prior to making dilutions.
3. The standard has to be used immediately after reconstitution and cannot be stored.

External standard dilution

1. Label 6 tubes, one for each standard point: S2, S3, S4, S5, S6, S7.
2. Prepare 1:2 serial dilutions for the standard curve as follows: Pipette 150 µL of Calibrator Diluent into each tube S2-S7.
3. Pipette 150 µL of reconstituted standard (serves as the highest standard S1, concentration of standard 1 = 200 pg/mL) into the first tube labeled S2, and mix (concentration of standard 2 = 100 pg/mL).
4. Pipette 150 µL of this dilution into the second tube, labeled S3 and mix thoroughly before the next transfer.
5. Repeat serial dilutions 4 more times thus creating the points of the standard curve (see Figure 6).

   Calibrator Diluent serves as blank.

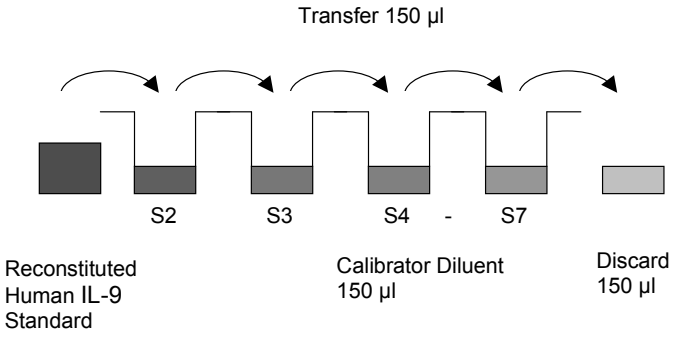

Fig. 6 External standard dilution

Test protocol

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 2–8°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.

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

	1	2	3	4
A	Standard 1 (200.0 pg/mL)	Standard 1 (200.0 pg/mL)	Sample 1	Sample 1
B	Standard 2 (100.0 pg/mL)	Standard 2 (100.0 pg/mL)	Sample 2	Sample 2
C	Standard 3 (50.0 pg/mL)	Standard 3 (50.0 pg/mL)	Sample 3	Sample 3
D	Standard 4 (25 pg/mL)	Standard 4 (25 pg/mL)	Sample 4	Sample 4
E	Standard 5 (12.5 pg/mL)	Standard 5 (12.5 pg/mL)	Sample 5	Sample 5
F	Standard 6 (6.3 pg/mL)	Standard 6 (6.3 pg/mL)	Sample 6	Sample 6
G	Standard 7 (3.1 pg/mL)	Standard 7 (3.1 pg/mL)	Sample 7	Sample 7
H	Blank	Blank	Sample 8	Sample 8

3. Add 50 µL Sample Diluent to all wells.
4. Add 50 µL extern diluted standard in duplicate to designated standard wells.
5. Add 50 µL of Calibrator Diluent in duplicate to the blank wells.

6. Add 50 µL of each sample in duplicate to the sample wells.
7. Prepare Biotin-Conjugate (see "Biotin-Conjugate" on page 3).
8. Add 50 µL of diluted Biotin-Conjugate to all wells, including the blank wells.
9. Cover with an adhesive film and incubate at room temperature (18–25°C) for 2 hours on a microplate shaker.
10. Prepare Streptavidin-HRP (see "Streptavidin-HRP" on page 3).
11. Remove adhesive film and empty wells. Wash microwell strips 4 times according to point 2 of the test protocol. 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 (18–25°C) for 1 hour on a microplate shaker.
14. Remove adhesive film and empty wells. Wash microwell strips 4 times according to point 2 of the test protocol. Proceed immediately to the next step.
15. Pipette 100 µL of TMB Substrate Solution to all wells.
16. Incubate the microwell strips at room temperature (18–25°C) for 30 minutes. Avoid direct exposure to intense light.

    The color development on the plate should be monitored and the substrate reaction 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.

    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 2–8°C in the dark.
18. Read absorbance of each microwell on a spectro-photometer 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.

Note: If instructions in this protocol have been followed, samples have not been diluted and the concentration read from the standard curve must not be multiplied by a dilution factor.

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 ordinate against the human IL-9 concentration on the abscissa. Draw a best fit curve through the points of the graph (a 5-parameter curve fit is recommended).
- To determine the concentration of circulating human IL-9 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 IL-9 concentration.
- If instructions in this protocol have been followed, samples have not been diluted and the concentration read from the standard curve must not be multiplied by a dilution factor.
- Calculation of samples with a concentration exceeding standard 1 may will result in incorrect, low human IL-9 levels. Such samples require further external predilution according to expected human IL-9 values with Sample Diluent in order to precisely quantitate the actual human IL-9 level.
- It is suggested that each testing facility establishes a control sample of known human IL-9 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.
- A representative standard curve is shown in Figure 7.

  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.

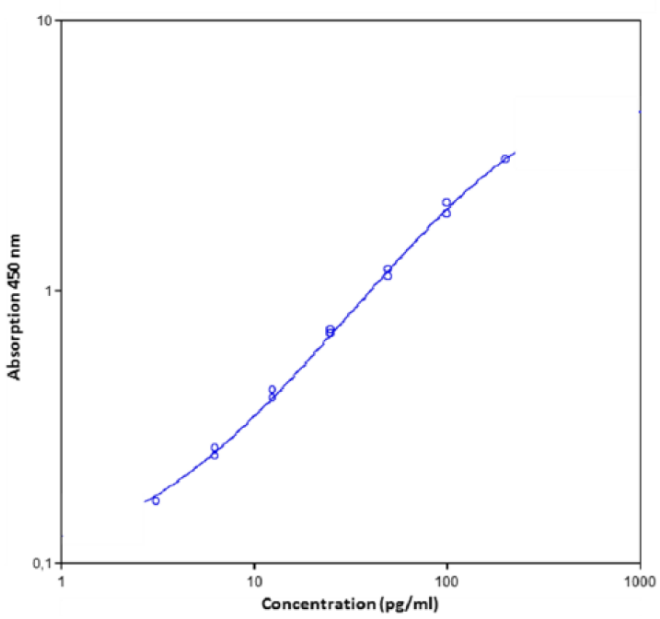

Fig. 7 Representative standard curve for human IL-9 ELISA. Human IL-9 was diluted in serial 2-fold steps in Calibrator Diluent.

Table 2 Typical data using the human IL-9 ELISA.

Measuring wavelength: 450 nm

Reference wavelength: 620 nm

Standard	human IL-9 Concentration (pg/mL)	O.D. at 450 nm	Mean O.D. at 450 nm	C.V. (%)
1	200.0	3.065 3.059	3.062	0.1
2	100.0	1.939 2.122	2.030	4.5
3	50.0	1.208 1.133	1.171	3.2
4	25.0	0.722 0.702	0.712	1.4
5	12.5	0.430 0.406	0.418	2.9
6	6.3	0.264 0.247	0.256	3.3
7	3.1	0.169 0.169	0.169	0.1
Blank	0.0	0.051 0.051	0.051	0.1

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.

Limitations

- Since exact conditions may vary from assay to assay, a standard curve must be established for every run.

- Bacterial or fungal contamination of either screen samples or reagents 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.

Performance characteristics

Sensitivity

The limit of detection of human IL-9 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 0.5 pg/mL (mean of 6 independent assays).

Reproducibility

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 IL-9. Two standard curves were run on each plate. Data below show the mean human IL-9 concentration and the coefficient of variation for each sample (see Table 3). The calculated overall intra-assay coefficient of variation was 5.3%.

Table 3 The mean human IL-9 concentration and the coefficient of variation for each sample.

Sample	Experiment	Mean human IL-9 concentration (pg/mL)	Coefficient of variation (%)
1	1	229.01	3.1
	2	222.35	1.6
	3	216.01	1.6
2	1	221.62	2.8
	2	212.26	4.2
	3	216.36	3.7
3	1	193.20	5.7
	2	169.31	7.1
	3	179.29	8.7
4	1	150.69	6.1
	2	126.78	8.6
	3	139.26	6.3
5	1	60.82	6.0
	2	60.36	7.0
	3	59.40	5.0
6	1	39.23	7.1
	2	35.45	5.2
	3	36.01	3.7
7	1	21.51	5.0
	2	19.63	8.7
	3	20.34	4.1
8	1	9.14	6.5
	2	8.09	6.5
	3	8.40	3.4

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 IL-9. Two standard curves were run on each plate. Data below show the mean human IL-9 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 4.8%.

Table 4 The mean human IL-9 concentration and the coefficient of variation of each sample.

Sample	Mean human IL-9 concentration (pg/mL)	Coefficient of variation (%)
1	222.46	2.9
2	216.75	2.2
3	180.60	6.6
4	138.91	8.6
5	60.19	1.2
6	36.90	5.5
7	20.50	4.6
8	8.54	6.4

Spike recovery

The spike recovery was evaluated by spiking 3 levels of human IL-9 into serum, plasma (EDTA, heparin, citrate), and cell culture supernatant. Recoveries were determined with 4 replicates each. The amount of endogenous human IL-9 in unspiked samples was subtracted from the spike values.

Sample matrix	Spike high (%)		Spike medium (%)		Spike low (%)	
	Mean	Range	Mean	Range	Mean	Range
Serum	108	91–119	86	70–98	86	86–87
Plasma (EDTA)	115	100–133	71	64–80	91	79–112
Plasma (citrate)	87	63–117	78	57–109	72	62–96
Plasma (heparin)	103	85–127	94	76–114	87	69–97
Cell culture supernatant	100	—	93	—	85	—

Dilution parallelism

Serum, plasma (EDTA, citrate, heparin), and cell culture supernatant samples with different levels of human IL-9 were analyzed at serial 2-fold dilutions with 4 replicates each.

Table 5 Dilution parallelism

Sample matrix	Dilution	Recovery of exp. val. (%)	
		Mean	Range
Serum	1:4	90	85–94
	1:8	91	89–93
	1:16	80	71–86
Plasma (EDTA)	1:4	104	99–112
	1:8	103	91–113
	1:16	99	86–109
Plasma (citrate)	1:4	83	85–91
	1:8	87	86–99
	1:16	83	80–96
Plasma (heparin)	1:4	97	85–116
	1:8	95	86–109
	1:16	95	80–112
Cell culture supernatant	1:4	98	–
	1:8	70	–
	1:16	70	–

Sample stability

Freeze-Thaw stability

Aliquots of serum samples (spiked) were stored at -20°C and thawed 3 times, and the human IL-9 levels determined. There was no significant loss of human IL-9 immunoreactivity detected by freezing and thawing.

Storage stability

Aliquots of serum samples (spiked) were stored at –20°C, 4°C, room temperature, and at 37°C, and the human IL-9 level determined after 24 hours. There was no significant loss of human IL-9 immunoreactivity detected during storage at -20°C and 4°C under above conditions.

A significant loss (–20%) of human IL-9 immunoreactivity was detected during storage room temperature and at 37°C after 24 hours.

Specificity

The assay detects both natural and recombinant human IL-9. The cross-reactivity and interference of circulating factors of the immune system was evaluated by spiking human IL-2, IL-4, IL-15, and IL-21 proteins at physiologically relevant concentrations into a human IL-9 positive sample. No cross-reactivity or interference was detected.

Expected values

Panels of 40 serum as well as plasma samples (EDTA, citrate, heparin) from randomly selected donors (males and females) were tested for human IL-9. There were no detectable human IL-9 levels found.

Reagent preparation summary

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

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

Biotin-Conjugate

Make a 1:100 dilution of Biotin-Conjugate with 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

Streptavidin-HRP

Make a 1:200 dilution of Streptavidin-HRP with Assay Buffer (1x).

Number of Strips	Streptavidin-HRP (mL)	Assay Buffer (1x) (mL)
1 - 6	0.03	5.97
1 - 12	0.06	11.94

Human IL-9 standard

Reconstitute lyophilized human IL-9 standard with Calibrator Diluent. (Reconstitution volume is stated on the label of the standard vial).

Test protocol summary

Note: If instructions in this protocol have been followed, samples have not been diluted and the concentration read from the standard curve must not be multiplied by a dilution factor.

1. Determine the number of microwell strips required.
2. Wash microwell strips twice with Wash Buffer.
3. Add 50 µL of Sample Diluent to the all wells.
4. Add 50 µL standard in duplicate to designated standard wells.
5. Add 50 µL of Calibrator Diluent in duplicate to the blank wells.
6. Add 50 µL of each sample in duplicate to the sample wells.
7. Prepare Biotin-Conjugate (see "Biotin-Conjugate" on page 3).
8. Add 50 µL diluted Biotin-Conjugate to all wells.
9. Cover microwell strips and incubate 2 hours at room temperature (18–25°C) on a microplate shaker.
10. Prepare Streptavidin-HRP.
11. Empty and wash microwell strips 4 times with Wash Buffer.
12. Add 100 µL diluted Streptavidin-HRP to all wells.
13. Cover microwell strips and incubate 1 hour at room temperature (18–25°C) on a microplate shaker.
14. Empty and wash microwell strips 4 times with Wash Buffer.
15. Add 100 µL of TMB Substrate Solution to all wells.
16. Incubate the microwell strips for about 30 minutes at room temperature (18–25°C)
17. Add 100 µL Stop Solution to all wells.
18. Blank microwell reader and measure color intensity at 450 nm.

Note: If instructions in this protocol have been followed, samples have not been diluted and the concentration read from the standard curve must not be multiplied by a dilution factor.

Customer and technical support

Visit **https://www.novusbio.com/support** for service and support information.

Limited product warranty

Novus Biologicals and/or its affiliate(s) warrant their products as set forth in Novus Biologicals' Terms and Conditions.
If you have any questions, please contact Novus Biologicals at **https://www.novusbio.com/support**.