NOVATEINBIO
Accelerates Research and Development

Porcine IL-12/IL-23 p40 ELISA kit

Catalog number: NB-E50014 (96 wells)

The kit is designed to quantitatively detect the levels of Porcine IL-12/IL-23 p40 in ***cell culture supernatants.***

FOR RESEARCH USE ONLY. NOT FOR DIAGNOSTIC OR THERAPEUTIC PURPOSES

Important notes

Before using this product, please read this manual carefully; after reading the subsequent contents of this manual, please note the following specially:

- The operation should be carried out in strict accordance with the provided instructions.
- Store the unused strips in a sealed foil bag at 2-8°C.
- Always avoid foaming when mixing or reconstituting protein solutions.
- Pipette reagents and samples into the center of each well, avoid bubbles.
- The samples should be transferred into the assay wells within 15 minutes of dilution.
- We recommend that all standards, testing samples are tested in duplicate.
- Using serial diluted sample is recommended for first test to get the best dilution factor.
- If the blue color develops too light after 20 minutes incubation with the substrate, it may be appropriate to extend the incubation time (Do not over-develop).
- Avoid cross-contamination by changing tips, using separate reservoirs for each reagent.
- Avoid using the suction head without extensive wash.
- Do not mix the reagents from different batches.
- Stop Solution should be added in the same order of the Substrate Solution.
- TMB developing agent is light-sensitive. Avoid prolonged exposure to the light.

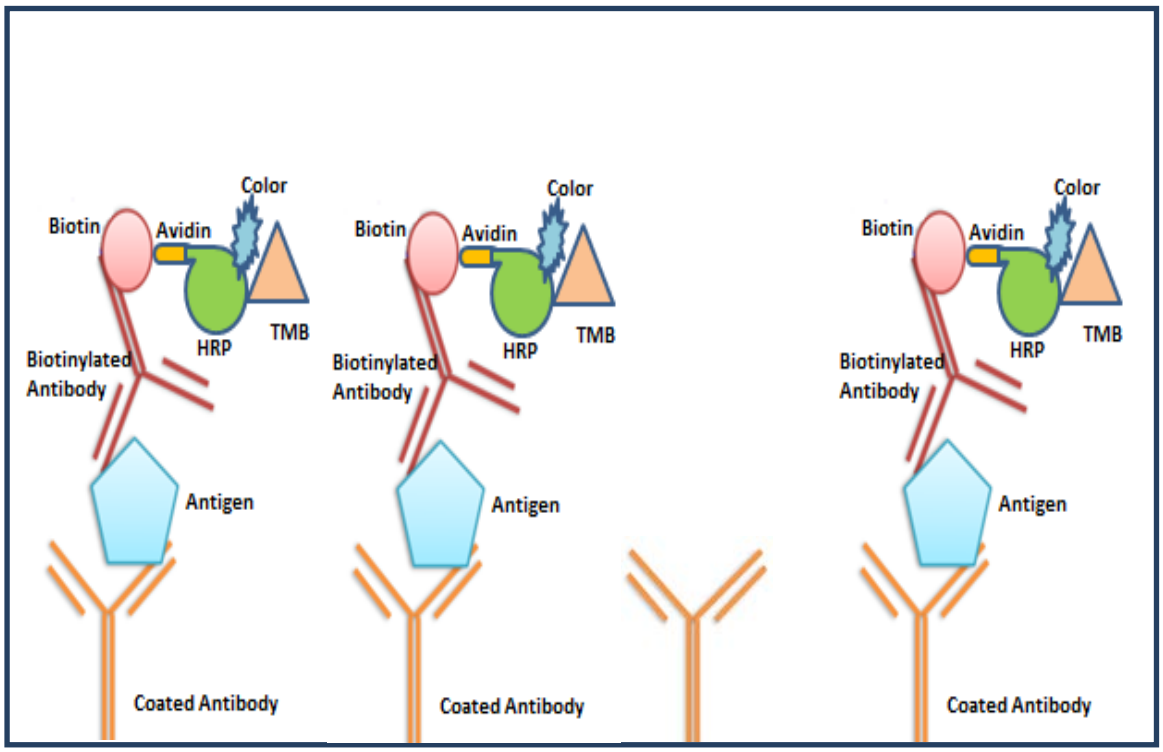
Intended use

The kit is used to quantify Porcine IL-12/IL-23 p40 in cell culture supernatants. It may work in detecting other samples, such as serum and plasma, but validation needs to be tested.

Standard range	78.1-5000 pg/ml
Sensitivity	5.0 pg/ml
Assay time	5 hours
Validity	Six months
Store at	2-8 °C

Assay principle

This Porcine IL-12/IL-23 p40 ELISA Kit is based on standard sandwich enzyme-linked immunosorbent assay technology. Porcine IL-12/IL-23 p40 specific antibody has been precoated onto 96-well plate. The test samples and the biotinylated Porcine IL-12/IL-23 p40 specific detection antibody are added to the wells subsequently and then followed by washing the plate. Streptavidin-HRP is added and unbound conjugates are washed away with Wash Buffer. HRP substrate TMB is used to visualize HRP enzymatic reaction. TMB is catalyzed by HRP to produce a blue color product that changes into yellow after adding acidic Stop Solution. The density of yellow is proportional to the Porcine IL-12/IL-23 p40 amount of sample captured in plate.



Materials supplied

1.Porcine IL-12/IL-23 p40 standard:	5ng/vialx2.
2. 96-well plate coated with anti-Porcine IL-12/IL-23 p40 Ab:	1.
3. Sample diluent buffer:	12 ml× 2.
4. Biotinylated Porcine IL-12/IL-23 p40 Ab:	1 vial.
5. Streptavidin-HRP:	1 vial.
6. Antibody diluent buffer:	12 ml.
7. Streptavidin-HRP diluent buffer:	12 ml.
8. Chromogenic solution A:	6 ml
9. Chromogenic solution B:	6 ml.
10. Stop Solution:	6 ml.
11. 20 × Wash Buffer:	25 ml.
12. Plate sealer	1.
13. Package insert	1.

Materials required but not supplied

- 37°C incubator.
- Standard plate reader capable of measuring absorbance at 450 nm.
- Adjustable pipettes and disposable pipette tips.
- Multi-channel pipettes, manifold dispenser or automated microplate washer.
- Distilled water.
- Absorbent paper.
- Materials used for sample preparation.
- Heat inactivated normal goat serum (for detection antibody dilution).

Sample Preparation and storage

- Cell culture supernatant: Remove particulates by centrifugation at 3000 x g for 10 minutes, analyze immediately or aliquot and store at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles. **The user should determine the optimal dilution factor**.
- Serum: Allow the serum to clot in a serum separator tube (about 4hours) at room temperature. Centrifuge at approximately 1000 X g for 15 min. Analyze the serum immediately or aliquot and store frozen at -20°C or below.

- Plasma: Collect plasma using heparin as an anticoagulant. Centrifuge for 15 min at 1000 x g within 30 minutes of collection. Analyze immediately or aliquot and store frozen at -20°C or below. EDTA and citrate are not recommended as the anticoagulant.

Note:

- Novateinbio is only responsible for the kit itself, but not for the samples consumed during the assay. The user should calculate the possible amount of the samples used in the whole test. Please reserve sufficient amount of samples in advance.
- Please predict the concentration before assaying. If values for these are not within the range of the standard curve, users must determine the optimal sample dilutions for their particular experiments.
- This kit is designed to detect Porcine IL-12/IL-23 p40 in cell culture supernatants, serum, plasma and other suitable sample solution. The users should check the expression profile of this protein before using this kit. If the samples are not specifically indicated in the manual, a preliminary experiment to determine the validity of the kit is necessary. We recommend that a serum and/or plasma sample as the positive control should be run in each assay.
- Owing to the possibility of mismatching between antigen from other resource and antibody used in our kits (e.g., antibody targets conformational epitope rather than linear epitope), some native or recombinant proteins from other manufacturers may not be recognized by our products.
- Cannot detect the samples containing NaN3, since NaN3 inhibits HRP (horseradish peroxidase) activity.
- Sample hemolysis will influence the result, so hemolytic specimen cannot be detected.

Fresh samples without long time storage is recommended for the test. Otherwise, protein degradation and denaturalization may occur in those samples and finally lead to wrong results.

Reagent Preparation.

Standard

- Porcine IL-12/IL-23 p40: Standard solution should be prepared no more than 2 hours prior to the experiment. Two tubes of standard are included in each kit. Use one tube for each experiment.
- 5000 pg/ml→78.1 pg/ml of Porcine IL-12/IL-23 p40 standard solutions:
- Add 1 ml of sample diluent into one standard tube. Keep the tube at room temperature for 10 minutes and mix thoroughly. This is 5000 pg/ml standard solution.
- Label 7 Eppendorf tubes with 5000 pg/ml, 2500 pg/ml, 1250 pg/ml, 625 pg/ml, 312.5

pg/ml, 156.2 pg/ml, and 78.1 pg/ml, respectively. This is 5000 pg/ml standard solution. 2-fold serial dilution from 5000 pg/ml to 78.1 pg/ml in seven 1.5 ml tubes.

- Make sure each tube has ≥ 300 µl of standard.

Note: The standard solutions are best used within 2 hours.

Biotinylated Porcine IL-12/IL-23 p40 antibody working solution

- The solution should be prepared no more than 2 hours prior to the experiment.
- The total volume should be 0.1ml/well x the number of wells (Allowing 0.1-0.2 ml more than total volume.
- Spin down before opening the vial. Biotinylated anti-Porcine IL-12/IL-23 p40 detection antibody should be diluted according to instructions on the vial label with Antibody diluent buffer. Allow the diluted Detection Antibody to sit at least 1-2 hours before use.

Streptavidin-HRP working solution

- The solution should be prepared no more than 1 hour prior to the experiment.
- The total volume should be 0.1ml/well x the number of wells (Allowing 0.1-0.2 ml more than total volume).
- Spin down before opening the vial. Streptavidin-HRP should be diluted according to instructions on the vial label with Streptavidin-HRP diluent buffer and mixed thoroughly.

Wash Buffer

- If crystals have formed in the 20 × wash buffer, warm to room temperature and mix gently until the crystals have completely dissolved.
- Dilute 25 ml Wash Buffer Concentrate (20 ×) to a total volume of 500ml with distilled water.

Assay procedures

Bring all reagents to room temperature before use. Porcine IL-12/IL-23 p40 Standard curve should be prepared for each experiment. The user will decide sample dilution factor by rough estimation of Porcine IL-12/IL-23 p40 concentration in samples.

1. Add 100 µl of sample or standards per well. Add 100 µl of the sample diluent into the control well (Zero well). Cover with an adhesive strip and incubate 2 hours at room temperature. Note: We recommend that each Porcine IL-12/IL-23 p40 standard solution and each sample is measured in duplicate.
2. Aspirate each well and wash with Wash Buffer, repeating the process two times for a total of three washes. Wash by filling each well with Wash Buffer (300 µl) using a squirt bottle, manifold dispenser, or auto-washer. Complete removal of liquid at each step is

essential for good performance. After the last wash, remove any remaining Wash Buffer by aspirating or by inverting the plate and blotting it against clean paper towels.

3. Add 100 µl of biotinylated Porcine IL-12/IL-23 p40 antibody working solution to each well. Cover with a new adhesive strip and incubate 2 hours at room temperature.
4. Repeat the aspiration/wash as in step 2.
5. Add 100 µl of the working solution of Streptavidin-HRP to each well. Cover the plate and incubate for 20 minutes at room temperature. Avoid placing the plate in direct light.
6. Repeat the aspiration/wash as in step 2 for five times.
7. Add 50 µl of Chromogenic solution A and 50 µl of Chromogenic solution B to each well. Cover and incubate for 10-15 minutes at room temperature (Protect from light. Do not over-develop).
8. Add 50 µl Stop Solution to each well. Mix well.
9. Read the Optical Density (O.D.) at 450 nm using a microtiter plate reader immediately.

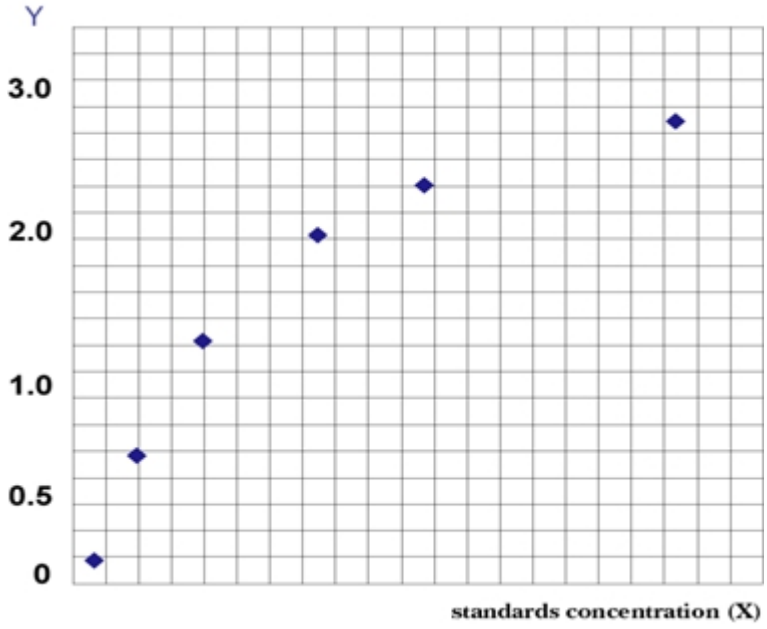
Result calculation

For calculation, (the relative O.D.450) = (the O.D.450 of each well) – (the O.D.450 of Zero well). The standard curve can be plotted as the relative O.D.450 of each standard solution (Y) vs. the respective concentration of the standard solution (X). The Porcine IL-12/IL-23 p40 concentration of the samples can be interpolated from the standard curve.

Note: if the samples measured were diluted, multiply the dilution factor to the concentrations from interpolation to obtain the concentration before dilution.

Typical data

This standard curve is for demonstration purpose only. A standard curve must be run with each assay.



Background

Interleukin 12 (IL-12), also known as NKSF or p70, is a 70-75 kDa heterodimeric glycoprotein belonging to the IL-12 family. IL-12 is encoded by two separate genes, IL-12A and IL-12B, in human. IL-12 is composed of two disulfide-linked subunits, p35 and p40. The mature p35 subunit is synthesized as a 197 amino acid (aa) containing seven cysteines and one potential N-linked glycosylation site. Mature human p35 shares 58% aa identity with mouse and rat p35. Mature human p40 is a 306 aa protein with 11 cysteines and three potential N-linked glycosylation sites. Mature human p40 is 66% aa identical to mouse and rat p40. IL-12 is produced in macrophages and dendritic cells, monocytes, Langerhans cells, neutrophils, keratinocytes, plasmacytoid dendritic cells, and microglia, etc. p35 mRNA is expressed by various cells and tissues, however, p35 have not been detected in culture supernatants of cells expressing only p35 or both p35 and p40 mRNAs. In cells expressing both p35 and p40 mRNAs, p40 mRNA is expressed at a higher level and free p40 is secreted together with heterodimeric IL-12 p70. Most of the free p40 secreted by the various human cell lines examined have been found to exist as monomers. The p40 and p35 by themselves do not have IL-12 activity, but the homodimer of p40 is capable of binding the IL-12 receptor and is an IL-12 antagonis.t

Human IL-12 receptor is composed of at least two type I transmembrane glycoproteins, Rβ1 and Rβ2. Rβ1 is the principal binding protein for the p40 , while Rβ2 might be the principal signal transduction component and serve as an attachment point for a disulfide-linked p35-p40 heterodimer. IL-12 plays an important role in regulating the activities of natural killer cells and T lymphocytes. It enhances cytotoxic activity and induces IFN-γ production in NK cells, T cells and dendritic epidermal T cells.

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