NOVATEINBIO
Accelerates Research and Development

Rat Interleukin 12, IL-12/P40 ELISA Kit

Catalog number: NB-E30537 (96 wells)

The kit is designed to quantitatively detect the levels of rat Interleukin 12 (IL-12/P40) in cell culture supernatant, serum, plasma and other suitable sample solutions.

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 15 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.

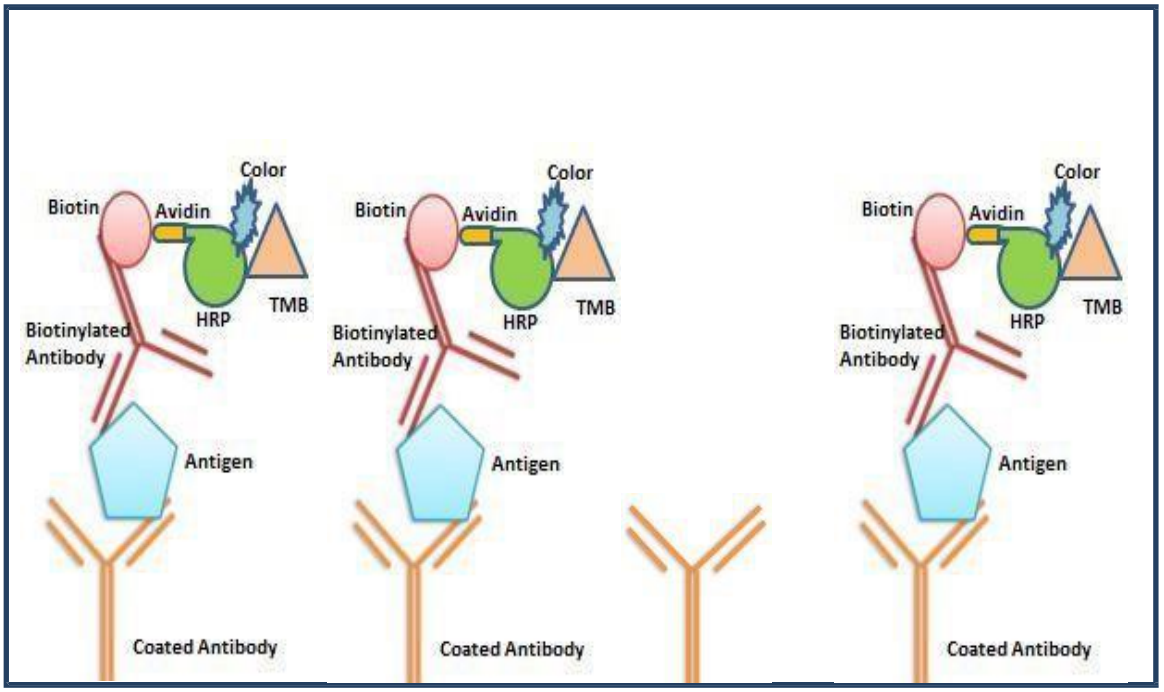
Intended use

The kit is used to quantify the rat IL-12/P40 in cell culture supernatant, serum, plasma and other suitable sample solution. This rat IL-12/P40 ELISA kit cannot discriminate between IL-12 P40 and IL-12-P70.

Standard range	7.8 –500 pg/ml
Assay time	3 h 40 min
Validity	Six months
Store at	2-8 °C

Assay principle

The rat IL-12/P40 ELISA Kit is based on standard sandwich enzyme-linked immunosorbent assay technology. Mouse anti-rat IL-12/P40 specific antibody has been pre-coated onto 96-well plate. Rat IL-12/P40 present in the standards/ samples binds to the capture antibody. Subsequently, biotinylated goat anti-rat IL-12/P40 detection antibody is added to form an Ab-Ag-Ab sandwich. Streptavidin-HRP is added and unbound conjugate is removed with wash Buffer. Next, addition of HRP substrate, TMB, results in the production of a blue colored product that changes to yellow after the addition of acidic Stop Solution. The density of yellow color is directly proportional to the amount of rat IL-12/P40 captured on plate.



Materials supplied

1. Rat IL-12/P40 standard:	1 ng/vial ×2
2. 96-well plate pre-coated with anti-rat IL-12/P40 Ab:	1
3. Sample Diluent buffer :	12 ml X 2
4. Detection antibody:	1 vial, dilution 1:100
5. Streptavidin-HRP:	1 vial dilution 1:100
6. Antibody Diluent Buffer	12 ml
7. Streptavidin-HRP Diluent Buffer	12 ml
8. Chromogen Solution A:	6 ml
9. Chromogen Solution B:	6 ml
10. Stop Solution:	6 ml
11. 20 × Wash Buffer:	25 ml
12. Plate sealer	2
13. Package insert	1

Materials required but not supplied

- Deionized water.
- 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.

Sample Preparation and storage

Store samples to be assayed within 24 hours at 2-8°C. For long-term storage, aliquot and freeze samples at -20°C. Avoid repeated freeze-thaw cycles.

- Cell culture supernatant, tissue lysate or body fluids: Remove particulates by centrifugation, analyze immediately or aliquot and store at -20°C
- 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.
- 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. EDTA and citrate are not recommended as the anticoagulant.

Reagent Preparation

Standard

- Rat IL-12/P40: Standard solution should be prepared no more than 2 hours prior to the experiment. Two tubes of standard (1 ng /vial) are included in each kit. Use one tube for each experiment.
- Prepare 500 pg/ml→7.8 pg/ml of rat IL-12/P40 standard solutions:
- Add 1 ml of Sample Diluent Buffer into one standard vial with 1 ng rat IL-12/P40 to prepare 1000 pg/ml standard solution. Keep the tube at room temperature for 10 minutes and mix thoroughly.
- Label 6 Eppendorf tubes with 500 pg/ml, 250 pg/ml, 125 pg/ml, 62.5 pg/ml, 31.25 pg/ml, 15.6 pg/ml, 7.8 pg/ml respectively. Then make 2-fold serial dilution from 1000 pg/ml to 15.6 pg/ml in 1.5 ml tubes with sample diluent buffer.
- Make sure each tube has ≥ 250 μl of standard.

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

Preparation of detection anti-rat IL-12/P40 antibody working solution

- The stock solution is stable at 2-8 ^{o}C for up to 6 months. After opening the vial, use within a month.
- The working solution should be prepared no more than 2 hours prior to the experiment
- The reagent is supplied as 100X concentrate. Empty the total contents in to 10.8 ml of Antibody Diluent Buffer or prepare the solution separately in a volume as needed. The solution should be mixed thoroughly.
- The total volume should be: 0.1 ml/well x the number of wells (Allowing 0.1-0.2 ml more than total volume.

Preparation of Streptavidin-HRP working solution

- The solution should be prepared no more than 1 hour prior to the experiment.
- The total volume should be: 0.1 ml/well x the number of wells (allowing 0.1-0.2 ml more than total volume).
- Streptavidin-HRP should be diluted 1:100 with Streptavidin-HRP Diluent buffer and mixed thoroughly.

Preparation of TMB developing agent working solution

Mix equal volumes of Chromogen Solution A and Chromogen Solution B. The total volume should be: 0.1 ml/well x the number of wells (allowing 0.1-0.2 ml more than total volume). Use it immediately (light sensitive reagent).

Wash Buffer

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

Assay Procedure

Bring all reagents to room temperature before use. Rat IL-12/P40 standard curve should be prepared for each experiment. The user will decide sample dilution factor by rough estimation of rat IL-12/P40 concentration in samples.

1. Add 100 µl of sample or standards per well. Add 0.1 ml of the sample diluent into the control well (Zero well). Cover with an adhesive strip and incubate 2 hours at room temperature (or over-night at 4 C for higher sensitivity).
   Note: We recommend that each rat IL-12/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 the Detection Antibody working solution to each well. Cover with a new adhesive strip and incubate for 1 hour 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 30 minutes at room temperature. Avoid placing the plate in direct light.
6. Repeat the aspiration/wash as in step 2 for three times.
7. Add 100 µl of TMB developing agent to each well. Cover and incubate at room temperature until a gradient develops and you see visible color in the lowest concentration well (approximately 10-25 minutes depending on the 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.

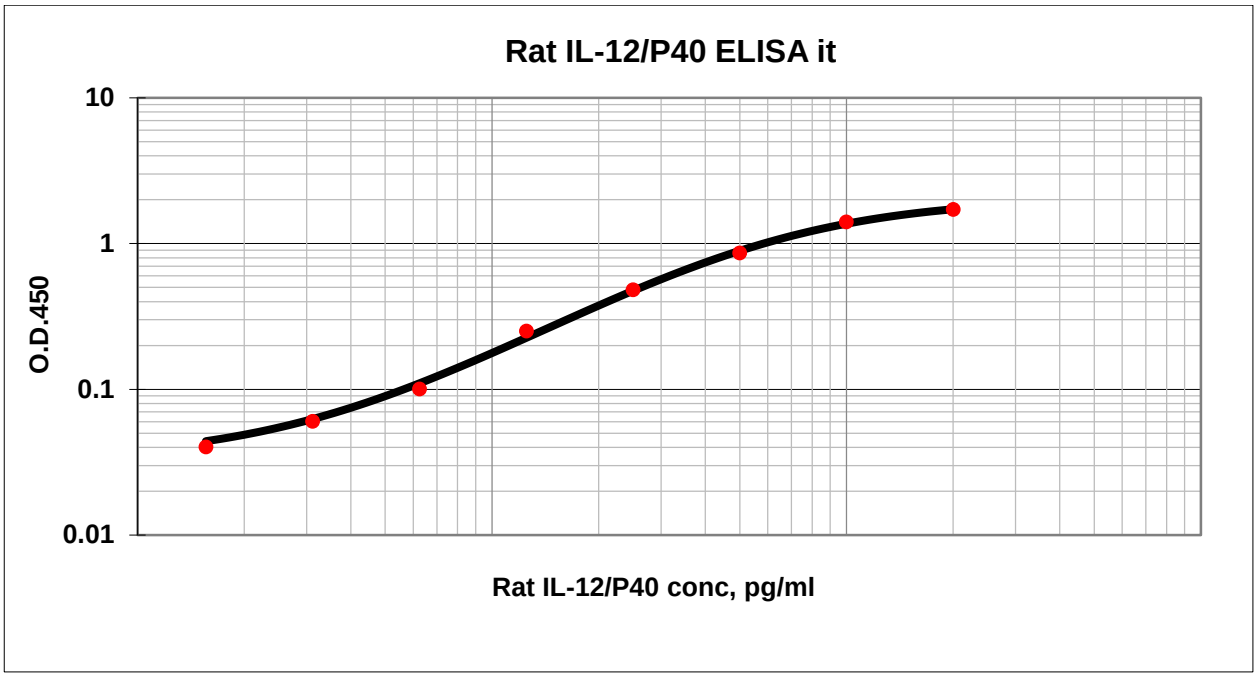
Result calculation

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 rat IL-12/P40concentration 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 was generated at the Novatein Biosciences laboratory for demonstration purpose only. A standard curve must be run with each assay.



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