

AviBion Human IL-12 ELISA Kit

User Manual

REF : IL12001

RUO  96

Regulatory Status: For research use only (RUO)

Please contact Orgenium's customer service representatives for inquiries, feedback or non-conforming products.



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1. INTENDED USE

Orgenium Laboratories' Interleukin-12 p70 (IL-12) ELISA is an enzyme-linked immunosorbent assay for the quantitative detection of human IL-12 p70 in cell culture supernatants, plasma (heparin and citrate), serum, urine and other body fluids.

2. INTRODUCTION

Interleukin-12 (IL-12) is a 70 kDa (p70) lymphokine. It is an important pro-inflammatory cytokine produced by dendritic cells, macrophages and B lymphocytes and it has been shown to have multiple effects on T cells and natural killer (NK) cells. IL-12 displays significant anti-tumor activity for a variety of tumor cell types by supporting the proliferation and clonal expansion of T-cells that specifically attack certain tumor types. *In vivo*, IL-12 appears to play a major role in auto-immune disease, in the resistance to bacterial and parasitic infections, and in antiviral responses including HIV.

Orgenium Laboratories' human IL-12 ELISA kit is an *in vitro* enzyme-linked immunosorbent assay for the quantitative measurement of human IL-12 in serum, plasma, cell culture supernatants and urine. This assay employs an antibody specific for human IL-12 coated on a 96-well plate. Standards, samples and biotinylated anti-human IL-12 are pipetted into the wells and IL-12 present in a sample is captured by the antibody immobilized to the wells and by the biotinylated IL-12 specific detection antibody. After washing away unbound biotinylated antibody, HRP-conjugated streptavidin is pipetted to the wells. The wells are again washed. Following this second wash step, TMB substrate solution is added to the wells, resulting in color development proportional to the amount of IL-12 bound. The Stop Solution changes the color from blue to yellow, and the intensity of the color is measured at 450 nm.



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3. CONTENTS OF THE KIT

Test components	Amount/Volume
<p>96 Well Plate with 12 Strips Break apart microtiter test strips each with IL-12 antibody coated single wells Ready for use.</p>	1 frame
<p>IL-12 Standard 1000 pg/ml Diluted & Stabilized Recombinant Human IL-12 (see label for stock concentration) Ready for use.</p>	1.5 ml
<p>Biotinylated IL-12 antibody. Ready for use.</p>	10 ml
<p>HRP-Conjugated Avidin. Ready for use.</p>	12 ml
<p>20x Wash Buffer concentrate (sufficient for 1000ml) Dilute 1:20</p>	50 ml
<p>Sample Diluent Ready for use</p>	100 ml
<p>Stop solution 2 N H₂SO₄ Ready for use.</p>	8 ml
<p>TMB-Substrate Ready for use</p>	8 ml

4. STORAGE AND STABILITY

Reagent	Storage	Stability
Antibody coated 96 well plates with 12 strips. Break apart microtiter test strips each with 8 antibody coated single wells	Store at 2-8°C in closed aluminum pouch with desiccant Strips which are not used must be stored in the re-sealable aluminum pouch in humidity free and airtight conditions!	3 months after opening
IL-12 Standard Ready for use	Store at 2-8°C	3 months after opening
Biotinylated antibody. Ready for use.	Store at 2-8°C <i>Avoid contamination</i> <i>(Use clean sterile tips)</i>	3 months after opening
HRP-Conjugated Avidin. Ready for use.	Store at 2-8°C <i>Avoid contamination</i> <i>(Use clean sterile tips)</i>	3 months after opening
Sample Diluent Ready for use.	Store at 2-8°C <i>Avoid contamination</i> <i>(Use clean sterile tips or pipettes)</i>	3 months after opening
20x Concentrated Wash Buffer Diluted Wash Buffer	Store at Room Temperature. 1x working dilution <i>Bottles used for the working dilution should be cleaned regularly, discard cloudy solutions</i>	Until expiry date at room temperature 5 working days at room temperature or 2 weeks at +4°C.
TMB-Substrate Solution Ready for use.	Ready for use solution at 2-8°C, protected from light! <i>Avoid contamination</i> <i>(Use clean sterile tips)</i>	Until expiry date (written on the bottle).
Stop Solution Ready for use.	Store at Room Temperature	Until expiry date at room temperature

5. ADDITIONAL MATERIALS REQUIRED

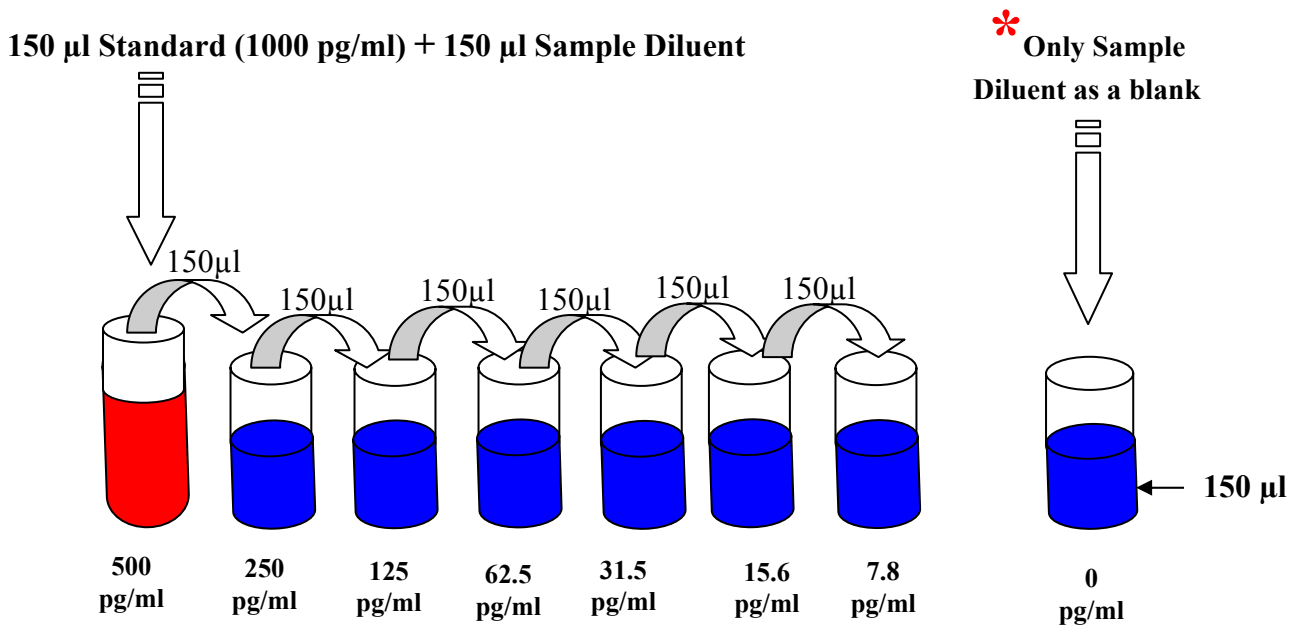
- Micro plate reader capable of measuring absorbance at 450 nm.
- Precision pipettes to deliver 2 µl to 1 ml volumes.
- Multi-channel pipet (25 µl to 350 µl).
- Adjustable 1-25 ml pipettes for reagent preparation.
- 100 ml and 1 liter graduated cylinders.
- Absorbent paper.
- Distilled or de-ionized water.
- Log-log graph paper or computer and software for ELISA data analysis.
- Tubes to prepare standard or sample dilutions.
- Timer

6. AMOUNTS OF THE REAGENTS NEEDED TO PERFORM THE TEST

No of strips used (with 8 well each)	Reagents				
	Biotinylated antibody 50 µl/well	Avidin-HRP 100µl/well	TMB substrate 50 µl/well	Stop Solution 25 µl/well	Wash Buffer 300 µl/well
1 (8 wells)	500µl	900 µl	500 µL	300 µl	30 ml
2 (16 wells)	1 ml	1.8 ml	1 ml	600 µL	55 ml
4 (32 wells)	2 ml	3.6 ml	2 ml	1.2 ml	110 ml
6 (48 wells)	3 ml	5.4 ml	3 ml	1.8 ml	165 ml
8 (64 wells)	4 ml	7.2 ml	4 ml	2.4 ml	220 ml
12 (96 wells)	6 ml	11 ml	6 ml	4 ml	350 ml

7. REAGENT AND SAMPLE PREPARATION

1. Bring all reagents and samples to room temperature (18-25°C) before use.
2. **Antibody coated plate:** Before opening the plastic pouch, determine the number of strips required to test samples plus standards and blanks in duplicate. Remove non-used strips from the plate-frame and restore them in the plastic pouch containing the desiccant for up to 1 month at 2-8°C.
3. **Dilution of test standard:**
 - a) Add 150 µl of IL-12 standard which contains 1000 pg/ml of IL-12 and 150 µl Sample Diluent to the first tube to obtain 500 pg/ml (Standard tube 1).
 - b) Add 150 µl of Sample Diluent to the other 5 tubes. Take 150 µl from the first tube (500 pg/ml) and start 2 fold serial dilutions in dilution tubes as described in the figure by mixing several times with the pipet in each tube (Total of 7 dilution tubes).
 - c) Sample Diluent serves as the zero standard (0 ng/ml) in tube 8 (use at least 2 wells as a test blank).



4. **Sample preparation and dilution:** Sample Diluent is used for dilution of serum/plasma samples, culture supernatants and urine samples requiring dilution. Store and dilute your samples in tubes or material made of polypropylene or any other tubes or plates with low binding surface.

Dilution of samples: Dilution of samples not required at initial screening. Samples that exceed the measuring range should be diluted 1:2 or 1:5 with sample diluent, and measured again. Samples with absorbance values >1.900 can be serially diluted 1:50, 1:100, 1:200, 1:400, 1:800 in sample diluent. The dilution factor must be taken in account when calculating the results.

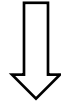
Serum, EDTA-anti-coagulated plasmas, cerebrospinal fluid and culture fluids are suitable for use in the assay (caution: separate plasma/serum and blood cells within 4 hours after collection, non-separated samples must be kept on temperatures from 2 to 8°C). Do not use grossly haemolyzed or lipemic specimens. If samples are to be run within 24 hours, they may be stored at 2-8°C; otherwise samples should be stored frozen (between -18 to -32°C, preferably $< -70^{\circ}\text{C}$). Up to 3 freeze-thaw cycles have no effect on the IL-12 levels of serum or plasma samples. Nonetheless, excessive freeze-thaw cycles should be avoided. Prior to the assay, frozen samples should be thawed as quickly as possible in tap water (18-25°C), do not use 37°C or 56°C water bath for this purpose.

5. **Wash Buffer:** If the 20x concentrated Wash Buffer contains visible crystals, warm it at 37°C and mix gently until dissolved. Dilute 25 ml of Wash Buffer Concentrate into de-ionized or distilled water to yield 500 ml of 1x Wash Buffer.
6. Vortex mix **Biotinylated antibody** solution gently before use.
7. Vortex mix **peroxidase (HRP) labeled streptavidin** gently before use.

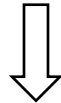
Caution: TMB substrate (Tetramethylbenzidine) and the Stop solution (H₂SO₄) are toxic or corrosive and should be handled with care. Use gloves during handling.

8. TEST PROCEDURE SUMMARY

1. Prepare all reagents, samples and standards.
Dilution of samples not required at initial screening.



2. Add 50 µl standard starting from 500 pg/mL or sample to each well.
3. Add 50 µl ready for use biotin antibody promptly to each well.
Incubate 1 hour 30 minutes at room temperature.



Wash 5x with 1x wash buffer

4. Add 100 µl ready for use HRP-Streptavidin solution.
Incubate 30 minutes at room temperature.



Wash 5x with 1x wash buffer

5. Add 50 µl TMB One-Step Substrate Reagent to each well.
Incubate 20 minutes at room temperature.



6. Add 25 µl Stop Solution to each well.
Read at 450 nm against *630 nm immediately.

**Correcting for optical imperfections in the microplates by subtracting $A_{630\text{ nm}}$ is recommended, but not an essential procedure.*



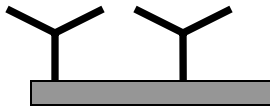
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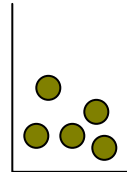
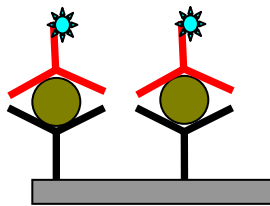


TEST PRINCIPLE



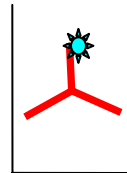
IL-12 Antibody coated test well

STEP 1



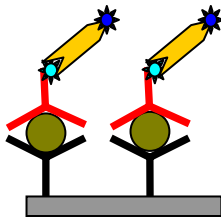
Add 50µL of IL-12 containing sample to test well

+



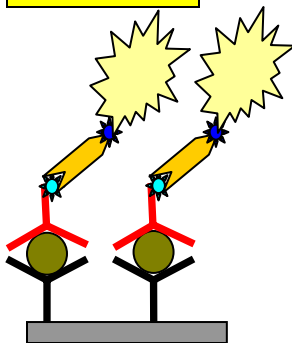
Add 50 µL of Biotinylated IL-12 antibody to test well

STEP 2

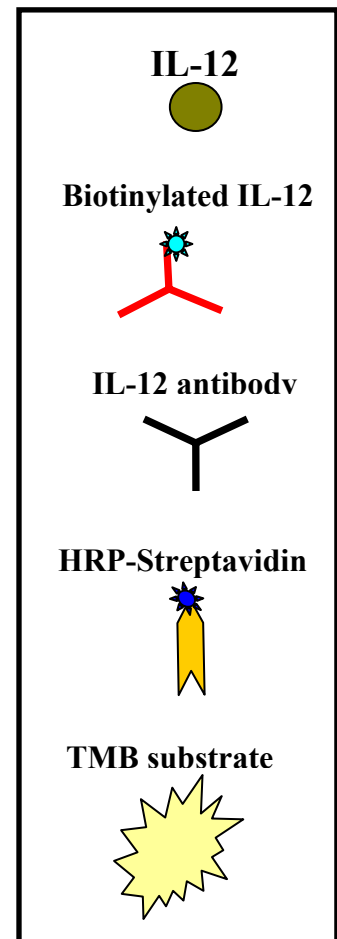


Add 100 µL of HRP-Streptavidin to test well

STEP 3



Add 50 µL of TMB substrate to test well



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9. PROCEDURAL NOTES/LAB QUALITY CONTROL

- When not in use, kit components should be refrigerated. All reagents should be warmed to room temperature before use.
- Microtiter plates should be allowed to come to room temperature before opening the foil pouches.
- Once the desired number of strips has been removed, immediately reseal the pouch and store at 2 - 8°C to maintain plate integrity. Protect from humidity.
- Samples should be collected in pyrogen/endotoxin-free tubes.
- Samples should be frozen if not analyzed shortly after collection. Avoid multiple freeze-thaw cycles of frozen samples. Thaw completely and mix well prior to analysis.
- When possible, avoid use of badly hemolyzed or lipemic sera. If large amounts of particulate matter are present, centrifuge or filter prior to analysis.
- It is recommended that all standards, controls and samples be run in duplicate.
- Samples that are >500 pg/mL should be diluted with Sample Diluent.
- When pipetting reagents, maintain a consistent order of addition from well-to-well. This ensures equal incubation times for all wells.
- Cover or cap all reagents when not in use.
- Do not use reagents after the kit expiration date.
- Read absorbances within 20 minutes of assay completion.
- In-house controls should be run with every assay. If control values fall outside pre-established ranges, the accuracy of the assay is suspect.
- All residual wash liquid must be drained from the wells by efficient aspiration or by decantation followed by tapping the plate forcefully on absorbent paper. **Never** insert absorbent paper directly into the wells.
- Because *TMB Chromogen* is light sensitive, avoid prolonged exposure to light. Also avoid contact between *Stabilized Chromogen* and metal, or color may develop.

10. ASSAY PROCEDURE

1. Bring all reagents and samples to room temperature (18 - 25°C) before use. It is recommended that all standards and samples be run at least in duplicate. Leave some wells as a reagent blank (2 to 4 wells).

FIRST STEP: STANDARD, SAMPLES AND BLANK+ BIOTINYLATED-ANTIBODY

2. Pipette 50 µl of Sample and 50 µl of each diluted standard starting from 500 pg/mL (see page 7) into appropriate wells. Pipette 50 µl of Sample diluent to the wells which will be used as a blank.

3. Add 50 µl of Green colored Biotinylated detection antibody to all wells containing standards and samples (total reaction volume is 100 µl). Tap the plate gently by hand to homogenize your mixture.

4. Incubate at room temperature for 1 hr 30 min. without shaking.

SECOND STEP: STREPTAVIDIN-HRP

5. Wash 5 times with 1x Wash Buffer (300 µl each).

To wash: Empty plate contents. Use a multi-channel pipette to fill each well with 300 µl of Wash Buffer, then empty plate contents again. Repeat procedure 4 additional times for a total of FIVE washes. Gently blot plate onto paper towels or other absorbent material.

Note: For automated washing, aspirate all wells and wash 5 times with Wash Buffer. Blot plate onto paper towels or other absorbent material. Never let reaction wells to dry. Continue to next step without delay or interruption.

6. Add 100 µl of prepared Streptavidin-HRP solution (Ready to use) to each well. Incubate for 30 minutes at room temperature.

THIRD STEP: TMB SUBSTRATE

7. Wash 5 times with 1x Wash Buffer (300 µl each).

8. Add 50 µl of TMB Ready to use Substrate Reagent to each well.

Incubate for 20 minutes at room temperature in the dark.

FOURTH STEP: STOP REACTION

9. Add 25 µl of Stop Solution to each well. Read at 450 nm within 15 minutes.

Correcting for optical imperfections in the microplates by subtracting $A_{630\text{ nm}}$ is recommended, but not an essential procedure.



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FIFTH STEP: READING AND CALCULATION

10. Calculate the mean of reagent blank absorbance values and subtract it from all test well values (standard and test samples). Mean reagent blank absorbance value at 450 nm should be less than 0.200.
11. Calculate your results against standard.

11. CALCULATION OF RESULTS

The standard curve has to be determined individually for each experiment. Correct each absorbance value of all standards by subtracting the O.D. value of the reagent blank (Bl = only sample diluent). Calculate the mean absorbance value for each standard from the duplicates.

The standard curve is used to determine the amount of IL-12 in an unknown sample. The standard curve is generated by plotting the average O.D. (450 nm) obtained for each of the standard concentrations on the vertical (Y) axis versus the corresponding IL-12 concentration (pg/mL) on the horizontal (X) axis.

Construct the standard curve using graph paper or statistical software.

If samples generate values higher than the highest standard, dilute the samples with Sample Diluent and repeat the assay, the concentration read from the standard curve must be multiplied by the dilution factor.

12. TYPICAL DATA

The following data were obtained for the various IL-12 standards over the range of 0 to 500 pg/mL.

Please note: This results are an example only. A standard curve should be generated each time the assay is performed. Do not use these values in your calculations.

Standard	Mean Optical Density (450 nm)
500 pg/mL	2.576
250 pg/mL	1.214
125 pg/mL	0.628
62.5 pg/mL	0.386
31.2 pg/mL	0.192
15.6 pg/mL	0.102
7.8 pg/mL	0.094
0 pg/mL (Sample Diluent)	0.045

****Mean sample diluent value should be less than 0.200***



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13. TEST PERFORMANCE

	IL-12
Assay range	7.8-500 pg/ml
Standard curve points	500, 250, 125, 62.5, 31.25, 15.62, 7.8 and 0 pg/ml.
Intra-Assay-Precision	≤6%
Inter-Assay-Precision	≤4%
Inter-Lot-Precision	≤8%
Cross-Reactivity	No cross reactivity was observed with the following recombinant human proteins: IIL-1α, IL-1β, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-13, TNF α, TARC
Interferences	No interferences to bilirubin up to 0.3 mg/mL, haemoglobin up to 8.0 mg/mL and triglycerides up to 5.0 mg/mL
Specificity	Recognizes both natural and recombinant human IL-12.
Sensitivity	<2 pg/ml.

14. REFERENCES

1. Tejde A, et al. Immune complex-stimulated production of interleukin-12 in peripheral blood mononuclear cells is regulated by the complement system. Clin. Exp. Immunol. 2004;137: 521.
2. Pekkari K, et al. Truncated thioredoxin (Trx80) induces production of interleukin-12 and enhances CD14 expression in human monocytes. Blood 2001;97: 3184.
3. Wong CK, Ho CY, Li EK, Lam CW. Elevation of proinflammatory cytokine (IL-18, IL-17, IL-12) and Th2 cytokine (IL-4) concentrations in patients with systemic lupus erythematosus. Lupus. 2000;9:589-93.
4. Cordero OJ, Salgado FJ, Mera-Varela A, Nogueira M. Serum interleukin-12, interleukin-15, soluble CD26, and adenosine deaminase in patients with rheumatoid arthritis. Rheumatol Int. 2001;21:69-74.

LIABILITY

This kit is intended for research use only by personnel trained and qualified to carry out diagnostic or research activities.

If the recipient of this kit passes it on in any way to a third party, this instruction must be enclosed, and said recipient shall at own risk secure in favor of Orgenium Laboratories all limitations of liability herein.

Orgenium Laboratories shall not be responsible for any damages or losses due to using the kit in any way other than as expressly stated in these Instructions.

The liability of Orgenium Laboratories shall in no event exceed the commercial value of the kit.

Orgenium Laboratories shall under no circumstances be liable for indirect, special or consequential damages, including but not limited to loss of profit.



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15. TROUBLESHOOTING

Problem	Cause	Solution
Poor standard Curve	<ol style="list-style-type: none"> 1. Inaccurate pipetting or pipetting error 2. Improper standard dilution 3. Bacterial contamination of reagents 	<p>Check pipettes and calibrate regularly.</p> <p>Vortex the stock before use and dilute carefully in an eppendorf tube.</p>
Low signal	<ol style="list-style-type: none"> 1. Shorter incubation than recommended 2. Inadequate reagent volumes or improper dilution or pipetting error 	<p>Ensure sufficient incubation time;</p> <p>Check pipettes and ensure correct performance.</p>
Large CV	Inaccurate pipetting and drying of wells during test procedure.	<p>Check pipettes</p> <p>Fill the wells promptly with wash buffer and reagents.</p>
High background	<ol style="list-style-type: none"> 1. Plate is insufficiently washed 2. Contaminated wash Buffer 3. Wash buffer volume is less than advised 	<p>Review the manual for proper wash. If using a plate washer, check that all ports are unobstructed and clean.</p> <p>Make a fresh wash buffer</p> <p>Use 300µl per well</p>
Low sensitivity	<ol style="list-style-type: none"> 1. Improper storage of the ELISA kit 2. Stop solution 3. Contamination of reagents 	<p>Store test kit components as advised in this user manual. Keep substrate solution protected from light.</p> <p>Stop solution should be added to each well before measure.</p> <p>Use clean sterile tips. Discard contaminated reagents.</p>