



## **Rat IL-12p40 ELISA Kit**

**Catalog Number**

**EK0521**

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**For the quantitative determination of rat interleukin 12p40 (IL-12p40) concentrations in cell culture supernates, serum, and plasma.**

**This instruction must be read in its entirety before using this product.  
For research use only. Not for use in diagnostic procedures.**

### **Contact information**

Tel: 1-301-446-2499

Fax: 1-301-446-2413

Email: [tech@signalwayantibody.com](mailto:tech@signalwayantibody.com) Web: [www.sabbiotech.com](http://www.sabbiotech.com)

## **PRINCIPLE OF THE ASSAY**

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for IL-12p40 has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any IL-12p40 present is bound by the immobilized antibody. Following incubation unbound samples are removed during a wash step, and then a detection antibody specific for IL-12p40 is added to the wells and binds to the combination of capture antibody-IL-12p40 in sample. Following a wash to remove any unbound combination, and enzyme conjugate is added to the wells. Following incubation and wash steps a substrate is added. A coloured product is formed in proportion to the amount of IL-12p40 present in the sample. The reaction is terminated by addition of acid and absorbance is measured at 450nm. A standard curve is prepared from seven IL-12p40 standard dilutions and IL-12p40 sample concentration determined.

## **DETECTION RANGE**

15.6 pg/ml - 1000 pg/ml

## **SENSITIVITY**

The minimum detectable dose was 7pg/mL.

## **SPECIFICITY**

This assay recognizes both natural and recombinant rat IL-12p40. The factors listed below were prepared at 50ng/ml in Standard /sample Diluent and assayed for cross-reactivity and no significant cross-reactivity or interference was observed.

Table 1: Factors assayed for cross-reactivity

<b>Recombinant human</b>	<b>Recombinant mouse</b>	<b>Recombinant rat</b>
IL-12p40	IL-12p40	IFN-
		TNF- $\alpha$ <input type="checkbox"/>
		IL-1 <input type="checkbox"/>
		IL-2
		IL-4
		IL-5

## **PRECISION**

The coefficient of variation of both intra-assay and inter-assay were less than 10%.

## **MATERIALS PROVIDED**

1. Aluminium pouches with a Microwell Plate coated with monoclonal antibody to rat IL-12p40 (8×12)
2. 2 vials rat IL-12p40 Standard lyophilized, 1000 pg/ml upon reconstitution
3. 2 vials concentrated Biotin-Conjugate anti-rat IL-12p40 antibody
4. 2 vials Streptavidin-HRP solution,
5. 1 bottle Standard /sample Diluent
6. 1 bottle Biotin-Conjugate antibody Diluent
7. 1 bottle Streptavidin-HRP Diluent
8. 1 bottle Wash Buffer Concentrate 20x (PBS with 1% Tween-20)
9. 1 vial Substrate Solution
10. 1 vial Stop Solution
11. 3 pieces Adhesive Films
12. package insert

## **STORAGE**

Table 2: Storage of the kit

<b>Unopened Kit</b>	Store at 2 - 8° C. Do not use past kit expiration date.	
<b>Opened/ Reconstituted Reagents</b>	Standard /sample Diluent	May be stored for up to 1 month at 2 - 8° C.**
	Concentrated Biotin-Conjugate	
	Streptavidin-HRP solution	
	Biotin-Conjugate antibody Diluent	
	Streptavidin-HRP Diluent	
	Wash Buffer Concentrate 20x	
	Substrate Solution	
	Stop Solution	

	Standard	Aliquot and store for up to 1 month at $\leq 20^{\circ}\text{C}$ . Avoid repeated freeze-thaw cycles. Diluted standard shall not be reused.
	Microplate Wells	Return unused wells to the foil pouch containing the desiccant pack, reseal along entire edge of zip-seal. May be stored for up to 1 month at $2 - 8^{\circ}\text{C}$ .**

\*\*Provided this is within the expiration date of the kit.

**THE REQUIRED ITEMS (not provided, but can help to buy):**

1. Microplate reader (450nm).
2. Micro-pipette and tips: 0.5-10, 2-20, 20-200, 200-1000ul.
3.  $37^{\circ}\text{C}$  incubator, double-distilled water or deionized water, coordinate paper, graduated cylinder.

**PRECAUTIONS FOR USE**

1. Store kit reagents between  $2^{\circ}\text{C}$  and  $8^{\circ}\text{C}$ . After use all reagents should be immediately returned to cold storage ( $2^{\circ}\text{C}$  to  $8^{\circ}\text{C}$ ).
2. Please perform simple centrifugation to collect the liquid before use.
3. To avoid cross contamination, please use disposable pipette tips.
4. The Stop Solution suggested for use with this kit is an acid solution. Wear eye, hand, face, and clothing protection when using this material. Avoid contact of skin or mucous membranes with kit reagents or specimens. In the case of contact with skin or eyes wash immediately with water.
5. Use clean, dedicated reagent trays for dispensing the washing liquid, conjugate and substrate reagent. Mix all reagents and samples well before use.
6. After washing microtiter plate should be fully pat dried. Do not use absorbent paper directly into the enzyme reaction wells.
7. Do not mix or substitute reagents with those from other lots or other sources. Do not use kit reagents beyond expiration date on label.

8. Each sample, standard, blank and optional control samples should be assayed in duplicate or triplicate.
9. Adequate mixing is very important for good result. Use a mini-vortexer at the lowest frequency or Shake by hand at 10min interval when there is no vortexer.
10. Avoid microtiter plates drying during the operation.
11. Dilute samples at the appropriate multiple, and make the sample values fall within the standard curve. If samples generate values higher than the highest standard, dilute the samples and repeat the assay.
12. Any variation in standard diluent, operator, pipetting technique, washing technique, incubation time and temperature, and kit age can cause variation in binding.
13. This method can effectively eliminate the interference of the soluble receptors, binding proteins and other factors in biological samples.

### **SAMPLE COLLECTION AND STORAGE**

1. **Cell Culture Supernates** - Remove particulates by centrifugation.
2. **Serum** - Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at approximately 1000 x g. Remove serum, avoid hemolysis and high blood lipid samples.
3. **Plasma** - Recommended EDTA as an anticoagulant in plasma. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection.
4. Assay immediately or aliquot and store samples at -20°C. Avoid repeated freeze-thaw cycles.
5. Dilute samples at the appropriate multiple (recommended to do pre-test to determine the dilution factor).

**Note: The normal rat serum or plasma samples are suggested to make a 1:2 dilution.**

### **REAGENT PREPARATION**

1. Bring all reagents to room temperature before use.
2. **Wash Buffer** - Dilute 10mL of Wash Buffer Concentrate into deionized or distilled water to prepare 200mL of Wash Buffer. If crystals have formed in the concentrate Wash Buffer, warm to room temperature and mix gently until the crystals have completely dissolved.

- Standard** - Reconstitute the Standard with 1.0mL of Standard /sample Diluent. This reconstitution produces a stock solution of 1000 pg /mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions.

Pipette 500 $\mu$ L of Standard/sample Diluent into the 500 pg/mL tube and the remaining tubes. Use the stock solution to produce a 2-fold dilution series (below). Mix each tube thoroughly and change pipette tips between each transfer. The 1000 pg/mL standard serves as the high standard. The Standard/ sample Diluent serves as the zero standard (0 pg/mL).

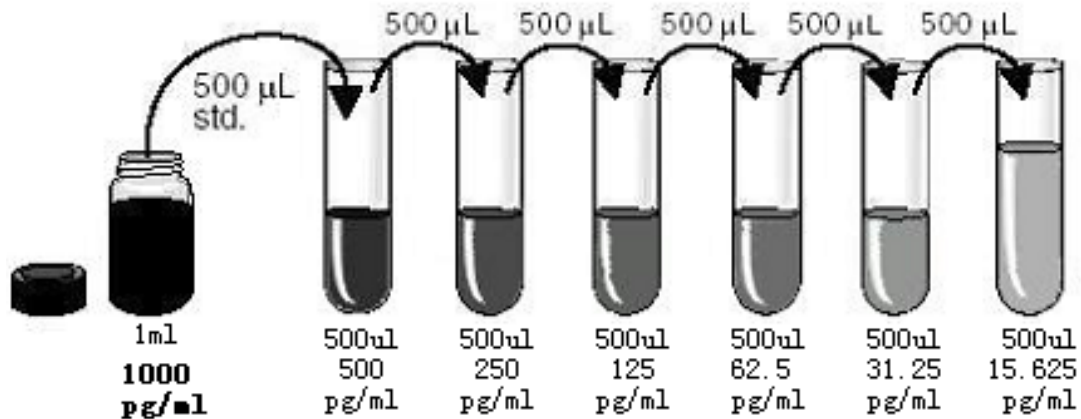
**If you do not run out of re-melting standard, store it at -20°C. Diluted standard shall not be reused.**

- Working solution of Biotin-Conjugate anti-rat IL-12p40 antibody: Make a 1:100 dilution of the concentrated Biotin-Conjugate solution with the Biotin-Conjugate antibody Diluent in a clean plastic tube.

**The working solution should be used within one day after dilution.**

- Working solution of Streptavidin-HRP: Make a 1:100 dilution of the concentrated Streptavidin-HRP solution with the Streptavidin-HRP Diluent in a clean plastic tube.

**The working solution should be used within one day after dilution.**



**Figure 1: Preparation of IL-12p40 standard dilutions**

### **GENERAL ELISA PROTOCOL**

- Prepare all reagents and working standards as directed in the previous sections.
- 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. Remove extra microwell strips from holder and store in foil bag with the desiccant provided at 2-8°C sealed tightly.

3. Add 100 $\mu$ L of Standard, control, or sample, per well, then add 50  $\mu$ L of the working solution of Biotin-Conjugate to each well. Cover with the adhesive strip provided and incubate 2 hours at RT. Adequate mixing is very important for good result. Use a mini-vortexer at the lowest frequency.
4. Aspirate each well and wash, repeating the process three times for a total of four washes. Wash by filling each well with Wash Buffer (350 $\mu$ L) using a squirt bottle, manifold dispenser or auto-washer. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
5. Add 100  $\mu$ L of the working solution of Streptavidin-HRP to each well. Cover with a new adhesive strip and incubate for 30 minutes at RT. Avoid placing the plate in direct light.
6. Repeat the aspiration/wash as in step 3.
7. Add 100  $\mu$ L of Substrate Solution to each well. Incubate for 10-20 minutes at RT. Avoid placing the plate in direct light.
8. Add 100  $\mu$ L of Stop Solution to each well. Gently tap the plate to ensure thorough mixing.
9. Determine the optical density of each well immediately, using a microplate reader set to 450 nm.(optionally 630nm as the reference wave length;610-650nm is acceptable)

## **ASSAY PROCEDURE SUMMARY**

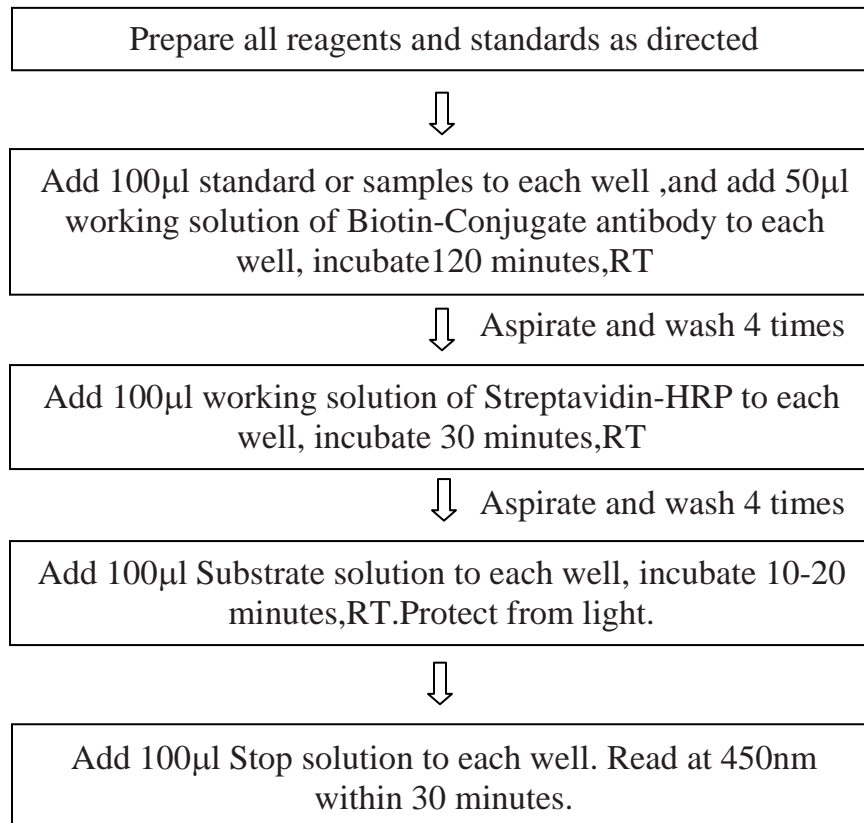


Figure 2: Assay procedure summary

### **TECHNICAL HINTS**

1. When mixing or reconstituting protein solutions, always avoid foaming.
2. To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.
3. To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
4. Substrate Solution should remain colorless until added to the plate. Stop Solution should be added to the plate in the same order as the



Substrate Solution. Keep Substrate Solution protected from light. Substrate Solution should change from colorless to gradations of blue.

5. A standard curve should be generated for each set of samples assayed. According to the content of tested factors in the sample, appropriate diluted or concentrated samples, it is best to do pre-experiment.

### **CALCULATION OF RESULTS**

1. Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density.
2. Create a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph.
3. The data may be linearized by plotting the log of the IL-12p40 concentrations versus the log of the O.D. and the best fit line can be determined by regression analysis. This procedure will produce an adequate but less precise fit of the data. If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.
4. This standard curve is provided for demonstration only. A standard curve should be generated for each set of samples assayed.

Table 3: Typical data using the IL-12p40 ELISA (Measuring wavelength:450nm, Reference wavelength:630nm)

Standard (pg/ml)	OD.	OD.	Average	Corrected
0	0.017	0.023	0.020	---
15.625	0.062	0.065	0.064	0.079
31.25	0.135	0.130	0.133	0.145
62.5	0.265	0.274	0.270	0.273
125	0.543	0.548	0.546	0.517
250	0.956	0.953	0.955	0.951
500	1.601	1.596	1.599	1.611
1000	2.093	2.096	2.095	2.093

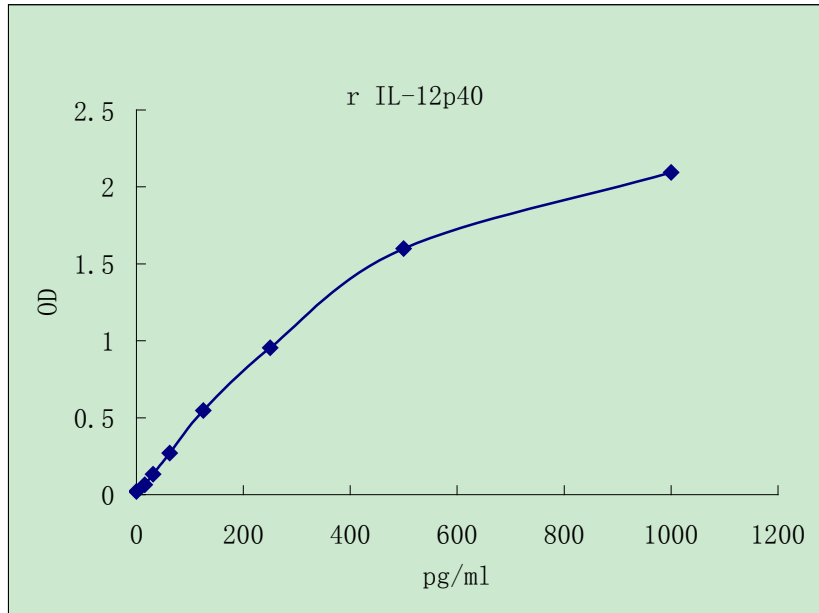


Figure 3: Representative standard curve for IL-12p40 ELISA. IL-12p40 was diluted in serial two-fold steps in Sample Diluent.

**Do not use this standard curve to derive test results. A standard curve must be run for each group of microwell strips assayed.**

### **BACKGROUND**

Interleukin 12(IL-12), also known as natural killer cell stimulatory factor (NKSF) or cytotoxic lymphocyte maturation factor (CLMF), is a pleiotropic cytokine originally identified in the medium of activated human B lymphoblastoid cell lines. IL-12 is produced by macrophages and B lymphocytes and has multiple effects on T-cells and NK cells, including stimulation of cytotoxic activity, proliferation, and promotion of Th1 development as well as IFN and TNF production. IL-12 is a disulfidelinked, 70 kDa(p70) heterodimeric glycoprotein composed of a 40 kDa (p40) subunit and a 35 kDa (p35) subunit. The p40 and p35 subunits by themselves have no IL-12 activity, the p40 dimer has been shown to bind the IL-12 receptor and to be an IL-12 antagonist. Free p35 has not been detected in supernatant solutions of cultured cells expressing only p35 or both p35 and p40 mRNAs. In contrast, p40 is secreted in excess of IL-12 in cells expressing both p35 and p40 mRNAs. The p40 subunit of IL-12 has been shown to have extensive amino acid sequence homology to the

extracellular domain of the human IL- 6 receptor while the p35 subunit shows distant but significant sequence similarity to IL-6, G-CSF, and chicken MGF. These observations have led to the suggestion that IL -12 might have evolved from a cytokine/soluble receptor complex. Human and mouse IL-12 share 70% and 60% amino acid sequence homology in their p40 and p35 subunits, respectively. IL-12 apparently shows species specificity with human IL-12 reportedly showing minimal activity in the murine system.

## **REFERENCES**

1. Kalka, P. et al (1997) J. Immunol. 159: 28..
2. Kalka, S. et al.(2000) Blood. 95:3183.
3. Temblay, J.N. et al.(2007) J Allergy Clin Immunol.120:659.