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ab203360 – IL-6 R alpha (Interleukin-6 Receptor alpha) Mouse SimpleStep ELISA® Kit

For the quantitative measurement of IL-6 R alpha (Interleukin-6 Receptor alpha) in mouse serum, plasma, urine, and cell culture supernatant.

This product is for research use only and is not intended for diagnostic use.

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1. Overview

Abcam's IL-6 R alpha (Interleukin-6 Receptor alpha) in vitro SimpleStep ELISA® (Enzyme-Linked Immunosorbent Assay) kit is designed for the quantitative measurement of IL-6 R alpha protein in mouse serum, plasma, urine, and cell culture supernatant.

The SimpleStep ELISA® employs an affinity tag labeled capture antibody and a reporter conjugated detector antibody which immunocapture the sample analyte in solution. This entire complex (capture antibody/analyte/detector antibody) is in turn immobilized via immunoaffinity of an anti-tag antibody coating the well. To perform the assay, samples or standards are added to the wells, followed by the antibody mix. After incubation, the wells are washed to remove unbound material. TMB substrate is added and during incubation is catalyzed by HRP, generating blue coloration. This reaction is then stopped by addition of Stop Solution completing any color change from blue to yellow. Signal is generated proportionally to the amount of bound analyte and the intensity is measured at 450 nm. Optionally, instead of the endpoint reading, development of TMB can be recorded kinetically at 600 nm.

Interleukin-6 Receptor (IL-6R, IL-6 Receptor), is part of a receptor that binds Interleukin-6. IL-6 Receptor weakly binds IL-6 with a low affinity, and then is presented to gp130 to facilitate transition into a high affinity signaling competent hexamer. Activation may lead to regulation of the immune response, acute-phase reactions and hematopoiesis. The expression of IL-6 Receptor has been implicated in the pathogenesis of many diseases, including multiple myeloma, auto immune diseases and prostate cancer. IL-6 Receptor has two isoforms. The first is a transmembrane protein in which the N-terminal extracellular portion can be released by proteolytic cleavage. The second isoform is a secreted form that lacks the transmembrane and intracellular portions. This assay is directed at the extracellular portion of IL-6 Receptor and detects both the soluble and membrane bound forms.

2. Protocol Summary

Remove appropriate number of antibody coated well strips. Equilibrate all reagents to room temperature. Prepare all reagents, samples, and standards as instructed.

Add standard or sample to appropriate wells.

Add Antibody Cocktail to all wells. Incubate at room temperature.

Aspirate and wash each well. Add TMB Substrate to each well and incubate.

Add Stop Solution at a defined endpoint.
Alternatively, record color development kinetically after TMB substrate addition.

3. Precautions

Please read these instructions carefully prior to beginning the assay.

- All kit components have been formulated and quality control tested to function successfully as a kit.
- We understand that, occasionally, experimental protocols might need to be modified to meet unique experimental circumstances. However, we cannot guarantee the performance of the product outside the conditions detailed in this protocol booklet.
- Reagents should be treated as possible mutagens and should be handled with care and disposed of properly. Please review the Safety Datasheet (SDS) provided with the product for information on the specific components.
- Observe good laboratory practices. Gloves, lab coat, and protective eyewear should always be worn. Never pipet by mouth. Do not eat, drink or smoke in the laboratory areas.
- All biological materials should be treated as potentially hazardous and handled as such. They should be disposed of in accordance with established safety procedures.

4. Storage and Stability

Store kit at +4°C immediately upon receipt. Kit has a storage time of 1 year from receipt, providing components have not been reconstituted.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in the Materials Supplied section.

5. Limitations

- Assay kit intended for research use only. Not for use in diagnostic procedures.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

6. Materials Supplied

Item	Quantity	Storage Condition
10X Mouse IL-6 R alpha Capture Antibody	600 µL	+2-8°C
10X Mouse IL-6 R alpha Detector Antibody	600 µL	+2-8°C
Mouse IL-6 R alpha Lyophilized Purified Protein	2 Vials	+2-8°C
Antibody Diluent 5BR	6 mL	+2-8°C
10X Wash Buffer PT	20 mL	+2-8°C
TMB Substrate	12 mL	+2-8°C
Stop Solution	12 mL	+2-8°C
Sample Diluent NS	50 mL	+2-8°C
Pre-Coated 96 Well Microplate (12 x 8 well strips)	96 Wells	+2-8°C
Plate Seal	1	+2-8°C

7. Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully perform this assay:

- Microplate reader capable of measuring absorbance at 450 or 600 nm.
- Method for determining protein concentration (BCA assay recommended).
- Deionized water.
- Multi- and single-channel pipettes.
- Tubes for standard dilution.
- Plate shaker for all incubation steps.
- Optional: Phenylmethylsulfonyl Fluoride (PMSF) (or other protease inhibitors).

8. Technical Hints

- This kit is sold based on number of tests. A 'test' simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.
- Samples generating values higher than the highest standard should be further diluted in the appropriate sample dilution buffers.
- Avoid foaming or bubbles when mixing or reconstituting components.
- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions.
- Ensure plates are properly sealed or covered during incubation steps.
- Complete removal of all solutions and buffers during wash steps is necessary to minimize background.
- As a guide, typical ranges of sample concentration for commonly used sample types are shown below in Sample Preparation (section 11).
- All samples should be mixed thoroughly and gently.
- Avoid multiple freeze/thaw of samples.
- Incubate ELISA plates on a plate shaker during all incubation steps.
- When generating positive control samples, it is advisable to change pipette tips after each step.
- The provided Antibody Diluents and Sample Diluents contain protease inhibitor aprotinin. Additional protease inhibitors can be added if required.
- To avoid high background always add samples or standards to the well before the addition of the antibody cocktail.

9. Reagent Preparation

- Equilibrate all reagents to room temperature (18-25°C) prior to use.
- The kit contains enough reagents for 96 wells. **The sample volumes below are sufficient for 48 wells (6 x 8-well strips); adjust volumes as needed for the number of strips in your experiment.**
- Prepare only as much reagent as is needed on the day of the experiment. Capture and Detector Antibodies have only been tested for stability in the provided 10X formulations.

9.1 1X Wash Buffer PT

Prepare 1X Wash Buffer PT by diluting 10X Wash Buffer PT with deionized water. To make 50 mL 1X Wash Buffer PT combine 5 mL 10X Wash Buffer PT with 45 mL deionized water. Mix thoroughly and gently.

9.2 Antibody Cocktail

Prepare Antibody Cocktail by diluting the capture and detector antibodies in Antibody Diluent 5BR. To make 3 mL of the Antibody Cocktail combine 300 µL 10X Capture Antibody and 300 µL 10X Detector Antibody with 2.4 mL Antibody Diluent 5BR. Mix thoroughly and gently.

10. Standard Preparation

- Always prepare a fresh set of standards for every use.
- Discard working standard dilutions after use as they do not store well.
- The following section describes the preparation of a standard curve for duplicate measurements (recommended).

10.1 **IMPORTANT:** If the protein standard vial has a volume identified on the label, reconstitute the IL-6 R alpha standard by adding that volume of Sample Diluent NS indicated on the label. Alternatively, if the vial has a mass identified, reconstitute the IL-6 R alpha standard by adding 500 µL Sample Diluent NS. Hold at room temperature for 10 minutes and mix gently. This is the 4,000 pg/mL **stock standard** Solution.

10.2 Label eight tubes, Standards 1– 8 and add 150 µL Sample Diluent NS to each tube.

10.3 Use the Stock Standard to prepare the following dilution series. Standard #8 contains no protein and is the Blank control:

Standard #	Volume to dilute (µL)	Volume Diluent (µL)	Mouse IL-6 R alpha (pg/mL)
1	150 µL Stock	150	2,000
2	150 µL Standard #1	150	1,000
3	150 µL Standard #2	150	500
4	150 µL Standard #3	150	250
5	150 µL Standard #4	150	125
6	150 µL Standard #5	150	62.5
7	150 µL Standard #6	150	31.3
8 (Blank)	N/A	300	0

11. Sample Preparation

TYPICAL SAMPLE DYNAMIC RANGE	
Sample Type	Range
Mouse Serum	0.03-0.5
Mouse Plasma (citrate)	0.03-0.5
Mouse Plasma (EDTA)	0.03-0.5
Mouse Plasma (heparin)	0.03-0.5
Mouse Urine	50
Cell Culture Supernatant	1-50

11.1 Plasma:

Collect plasma using citrate, EDTA or heparin. Centrifuge samples at 2,000 x g for 10 minutes. Dilute samples into Sample Diluent NS and assay. Store un-diluted plasma samples at -20ºC or below for up to 3 months. Avoid repeated freeze-thaw cycles.

11.2 Serum:

Samples should be collected into a serum separator tube. After clot formation, centrifuge samples at 2,000 x g for 10 minutes and collect serum. Dilute samples into Sample Diluent NS and assay. Store un-diluted serum at -20ºC or below. Avoid repeated freeze-thaw cycles.

11.3 Cell Culture Supernatants:

Centrifuge cell culture media at 2,000 x g for 10 minutes to remove debris. Collect supernatants and assay or dilute samples into Sample Diluent NS and assay. Store samples at -20°C or below. Avoid repeated freeze-thaw cycles.

11.4 Urine:

Centrifuge urine at 2,000 x g for 10 minutes to remove debris. Collect supernatants, dilute in Sample Diluent NS and assay. Store samples at -20°C or below. Avoid repeated freeze-thaw cycles.

12. Plate Preparation

- The 96 well plate strips included with this kit are supplied ready to use. It is not necessary to rinse the plate prior to adding reagents.
- Unused plate strips should be immediately returned to the foil pouch containing the desiccant pack, resealed and stored at 4°C.
- For each assay performed, a minimum of two wells must be used as the zero control.
- For statistical reasons, we recommend each sample should be assayed with a minimum of two replicates (duplicates).
- Differences in well absorbance or "edge effects" have not been observed with this assay.

13. Assay Procedure

- Equilibrate all materials and prepared reagents to room temperature prior to use.
- We recommend that you assay all standards, controls and samples in duplicate.

13.1 Prepare all reagents, working standards, and samples as directed in the previous sections.

13.2 Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, reseal and return to 4°C storage.

13.3 Add 50 µL of all samples and standards to appropriate wells.

13.4 Add 50 µL of the Antibody Cocktail to each well.

13.5 Seal the plate and incubate for 1 hour at room temperature on a plate shaker set to 400 rpm.

13.6 Wash each well with 3 x 350 µL 1X Wash Buffer PT. Wash by aspirating or decanting from wells then dispensing 350 µL 1X Wash Buffer PT into each well. Complete removal of liquid at each step is essential for good performance. After the last wash invert the plate and blot it against clean paper towels to remove excess liquid.

13.7 Add 100 µL of TMB Development Solution to each well and incubate for 10 minutes in the dark on a plate shaker set to 400 rpm.

Given variability in laboratory environmental conditions, optimal incubation time may vary between 5 and 20 minutes.
Note: The addition of Stop Solution will change the color from blue to yellow and enhance the signal intensity about 3X. To avoid signal saturation, proceed to the next step before the high concentration of the standard reaches a blue color of O.D.600 equal to 1.0.

13.8 Add 100 µL of Stop Solution to each well. Shake plate on a plate shaker for 1 minute to mix. Record the OD at 450 nm. This is an endpoint reading.

Alternative to 13.7 – 13.8: Instead of the endpoint reading at 450 nm, record the development of TMB Substrate kinetically. Immediately after addition of TMB Development Solution begin

recording the blue color development with elapsed time in the microplate reader prepared with the following settings:

Mode:	Kinetic
Wavelength:	600 nm
Time:	up to 20 minutes
Interval:	20 sec - 1 minute
Shaking:	Shake between readings

Δ Note: that an endpoint reading can also be recorded at the completion of the kinetic read by adding 100 µL Stop Solution to each well and recording the OD at 450nm.

13.9 Analyze the data as described below.

14. Calculations

- Subtract average of the zero standard absorbance measurement from all readings.
- Determine the average of the duplicate readings of the standards and plot against their concentrations.
- Draw the best smooth curve through these points to construct a standard curve.
- Most plate reader software or graphing software can plot these values and curve fit. A four parameter algorithm (4PL) usually provides the best fit, though other equations can be examined to see which provides the most accurate fit (e.g. linear, semi-log, log/log, 4 parameter logistic).
- Interpolate protein concentrations for unknown samples from the equation of the line of the standard curve plotted. Samples producing signals greater than that of the highest standard should be further diluted and reanalyzed, then multiplying the concentration found by the appropriate dilution factor.

15. Typical Data

Typical standard curve – Data provided is for demonstration purposes only. A new standard curve must be generated for each assay performed.

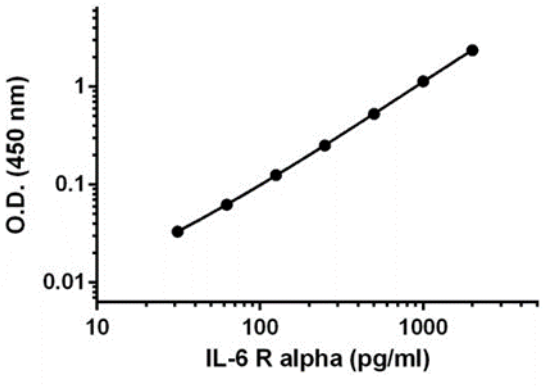

Figure 1. Example of RBP4 standard curve prepared in Sample Diluent NS. The RBP4 standard curve was prepared as described in Section 10. Raw data values are shown in the table. Background-subtracted data values (mean +/- SD) are graphed.

Standard Curve Measurements			
Conc. (pg/mL)	O.D. 450 nm		Mean O.D.
	1	2	
0	0.056	0.058	0.057
31.3	0.091	0.090	0.090
62.5	0.119	0.120	0.120
125	0.181	0.184	0.183
250	0.303	0.316	0.309
500	0.564	0.613	0.588
1,000	1.177	1.209	1.193
2,000	2.408	2.443	2.426

Figure 1: Example of IL-6 R alpha standard curve. The IL-6 R alpha standard curve was prepared as described in Section 10. Raw data values are shown in the table. Background-subtracted data values (mean +/- SD) are graphed.

16. Typical Sample Values

SENSITIVITY –

The calculated minimal detectable dose (MDD) is 12.6 pg/mL. The MDD was determined by calculating the mean of zero standard replicates (n=17) and adding 2 standard deviations then extrapolating the corresponding concentration.

RECOVERY –

Three concentrations of IL-6 R alpha were spiked in duplicate to the indicated biological matrix to evaluate signal recovery in the working range of the assay.

Sample Type	Average % Recovery	Range (%)
0.5% Mouse Serum	105	103-106
0.5% Mouse Plasma - Citrate	104	100-107
0.5% Mouse Plasma - EDTA	103	102-103
0.5% Mouse Plasma - Heparin	103	101-105
0.5% Mouse Urine	85	80-89
10% Cell Culture Media	88	84-93

LINEARITY OF DILUTION –

Linearity of dilution is determined based on interpolated values from the standard curve. Linearity of dilution defines a sample concentration interval in which interpolated target concentrations are directly proportional to sample dilution.

Native IL-6 R alpha was measured in the following biological samples in a 2-fold dilution series. Sample dilutions are made in Sample Diluent NS. Recombinant IL-6 R alpha was spiked into mouse urine diluted in a 2-fold dilution series in Sample Diluent NS.

Dilution Factor	Interpolated value	0.5% Mouse Serum	1% Mouse Plasma (Citrate)	1% Mouse Plasma (EDTA)	0.5% Mouse Plasma (Heparin)
Undiluted	pg/mL	108.6	210.7	195.1	95.8
	% Expected value	**100**	**100**	**100**	**100**
2	pg/mL	49.7	92.6	86.3	43.6
	% Expected value	**92**	**88**	**88**	**91**
4	pg/mL	23.8	44.6	41.8	20.2
	% Expected value	**88**	**85**	**86**	**84**
8	pg/mL	12.8	22.7	19.7	11.1
	% Expected value	**94**	**86**	**81**	**93**
16	pg/mL	7.29	**12.6**	**10.2**	**NL**
	% Expected value	**107**	**95**	**84**	**NL**

NL – Non linear

Dilution Factor	Interpolated value	50% Mouse Urine	50% J774A.1 Supernatant
Undiluted	pg/mL	840.5	115.7
	% Expected value	**100**	**100**
2	pg/mL	438.5	58.7
	% Expected value	**104**	**102**
4	pg/mL	230.8	26.2
	% Expected value	**110**	**91**
8	pg/mL	110.2	12.4
	% Expected value	**105**	**86**
16	pg/mL	**49.5**	**5.8**
	% Expected value	**94**	**80**

PRECISION –

Mean coefficient of variations of interpolated values from 3 concentrations of RBP4 within the working range of the assay.

	Intra-assay	Inter-Assay
n=	5	3
CV (%)	1.8	12

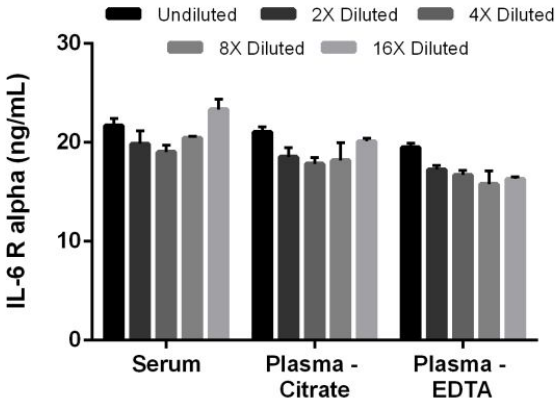

Figure 2: Interpolated concentrations of IL-6 R alpha in mouse serum, plasma (citrate), and plasma (EDTA). The concentrations of IL-6 R alpha were measured in duplicate and interpolated from the IL-6 R alpha standard curve and corrected for sample dilution. The interpolated dilution factor corrected values are plotted (mean +/- SD, n=2). The mean IL-6 R alpha concentration was determined to be 20.9 ng/mL in mouse serum, 19.1 ng/mL in mouse plasma (Citrate), and 17.1 ng/mL in mouse plasma (EDTA).

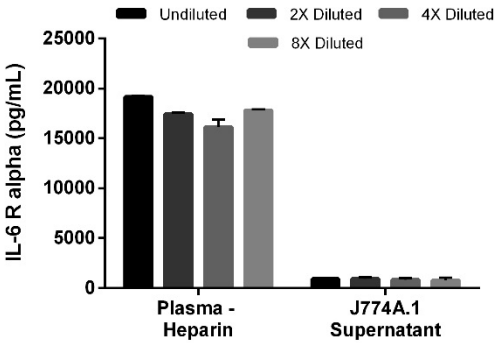

Figure 3: Interpolated concentrations of IL-6 R alpha in mouse plasma (heparin), and J774A.1 cell culture supernatant. The concentrations of IL-6 R alpha were measured in duplicate and interpolated from the IL-6 R alpha standard curve and corrected for sample dilution. The interpolated dilution factor corrected values are plotted (mean +/- SD, n=2). The mean IL-6 R alpha concentration was determined to be 17,650 pg/mL in mouse plasma (heparin), and 849 pg/mL in J774A.1 cell culture supernatant.

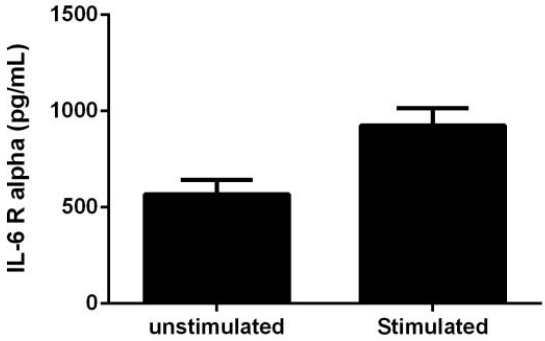

Figure 4: Comparison of secreted IL-6 R alpha in unstimulated and PMA/PHA-stimulated J774A.1 Cells. J774A.1 cells were grown in the absence (unstimulated) or presence of Phorbol Myristate Acetate (PMA) and phytohemagglutinin (PHA) (stimulated) for 3 days. IL-6 R alpha was measured in 2-fold diluted cell culture supernatants of unstimulated and PMA/PHA stimulated J774A.1 and cell culture media. Measured values were interpolated from the IL-6 R alpha Standard Curve diluted in Sample Diluent NS and corrected for dilution factor. Mean of duplicate values +/-SD are graphed, 568 pg/mL unstimulated, 849 pg/mL stimulated, and undetectable in media.

17. Assay Specificity

This kit recognizes both native and recombinant mouse IL-6 R alpha protein in serum, plasma, urine, and cell culture supernatant samples only.

Cell and tissue extract samples have not been tested with this kit

INTERFERENCE

Recombinant mouse IL-6 was prepared at 10, 100 and 250 pg/mL in Sample Diluent NS in the presence or absence of 1,000 pg/mL IL-6 R alpha and assayed for cross reactivity and interference. No cross reactivity was observed. No interference was observed with 10 pg/mL IL-6, but 22% and 49% interference was observed at 100 pg/mL and 250 pg/mL IL-6 respectively. Because in normal physiological conditions soluble IL-6 R alpha is expected to be >1,000-fold more abundant than IL-6, this level of interference is not biologically relevant.

18. Species Reactivity

This kit recognizes mouse IL-6 R alpha protein.

CROSS REACTIVITY

Recombinant human IL-6 R alpha and IL-6 were prepared at 50 ng/mL in Sample Diluent NS and assayed for cross reactivity and interference. No cross-reactivity or interference was observed.

Other species reactivity was determined by measuring 5% serum samples of various species, interpolating the protein concentrations from the mouse standard curve, and expressing the interpolated concentrations as a percentage of the protein concentration in mouse serum assayed at the same dilution.

Reactivity < 3% was determined for the following species:

- Human
- Rat
- Hamster
- Guinea Pig
- Rabbit
- Dog
- Goat
- Pig
- Cow

Please contact our Scientific Support team for more information

19. Troubleshooting

Problem	Reason	Solution
Poor standard curve	Inaccurate pipetting	Check pipettes
	Improper standard dilution	Prior to opening, briefly spin the stock standard tube and dissolve the powder thoroughly by gentle mixing
Low signal	Incubation times too brief	Ensure sufficient incubation times; increase to 2 or 3 hours standard/sample incubation
	Inadequate reagent volumes or improper dilution	Check pipettes and ensure correct preparation
	Incubation times with TMB too brief	Ensure sufficient incubation time until blue color develops prior addition of Stop solution
Large CV	Plate is insufficiently washed	Review manual for proper wash technique. If using a plate washer, check all ports for obstructions.
	Contaminated wash buffer	Prepare fresh wash buffer
Low sensitivity	Improper storage of the ELISA kit	Store your reconstituted standards at -80°C, all other assay components 4°C. Keep TMB substrate solution protected from light.
Precipitate in Diluent	Precipitation and/or coagulation of components within the Diluent.	Precipitate can be removed by gently warming the Diluent to 37°C.

20. Notes

Technical Support

For all technical or commercial enquiries please go to:

www.abcam.com/contactus
www.abcam.cn/contactus (China)
www.abcam.co.jp/contactus (Japan)