ab119541 – IL-2 Monkey ELISA Kit

Instructions for Use

For the quantitative measurement of monkey IL-2 concentrations in cell culture supernatant, serum and plasma (EDTA, citrate, heparin), amniotic fluid, urine and whole blood

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

Version 5 Last Updated 12 May 2020

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1. BACKGROUND

Abcam's IL-2 Monkey *in vitro* ELISA (Enzyme-Linked Immunosorbent Assay) kit is designed for accurate quantitative measurement of Monkey IL-2 concentrations in cell culture supernatant, serum and plasma (EDTA, citrate, heparin), amniotic fluid, urine and whole blood.

IL-2 specific antibodies have been precoated onto 96-well plates. Standards and test samples are added to the wells along with a biotin-conjugated IL-2 detection antibody then incubated at room temperature. Following washing, a Streptavidin-HRP conjugate is added to each well, incubated at room temperature and washed. TMB is added and then catalyzed by HRP to produce a blue color product that changes into yellow after the addition of an acidic stop solution. The density of yellow coloration is directly proportional to the amount of IL-2 captured on the plate.

IL-2 plays a central role in the activation and proliferation of lymphocytes that have been primed by antigens. IL-2 plays a pivotal role in for the expansion of most T-cells, natural killer cells and B-cells during certain phases of their response.

IL-2 is a 15 kDa glycoprotein encoded by a single gene located in the q26-28 region of Monkey chromosome 4. The cDNA deduced polypeptide consists of 153 amino acids.

IL-2 gene expression is regulated at the transcriptional level by several activation pathways. Antigen-specific proliferation of helper and cytotoxic T-lymphocytes following stimulation is critically dependent on IL-2 expression, secretion, and binding to receptors for IL-2 induced in an autocrine fashion on the surface of T-cells.

Apart from its most important role to mediate antigen-specific T-lymphocyte proliferation, IL-2 modulates the expression of interferon-

gamma and major histocompatibility antigens, stimulates proliferation and differentiation of activated B-cells, augments natural killer cell activity and inhibits granulocyte-macrophage colony formation.

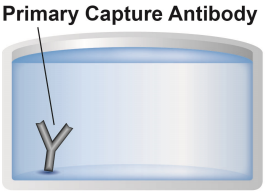
Alterations in the ability of T-cells to synthesize IL-2 have been observed in physiologic and pathologic states.

Because of the central role of IL-2 in immune response, IL-2 turned out to be a very important molecule for diagnostic and therapeutic implications.

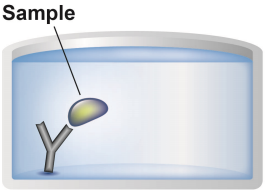
IL-2 displays antitumoral effects, thus being used in cancer therapy.

Monitoring of IL-2 levels in serum provides more detailed insights in several pathological situations such as cancer, infectious diseases, transplant rejection, multiple sclerosis, rheumatoid arthritis, systemic lupus erythematosus and type I diabetes.

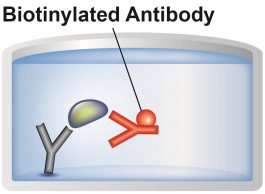
2. ASSAY SUMMARY



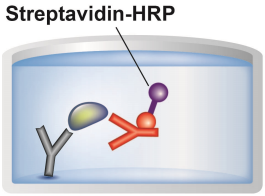
Prepare all reagents, samples and standards as instructed.



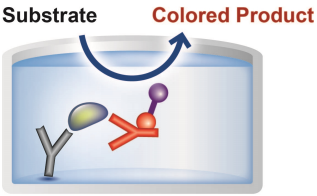
Add standards or samples to each well used.



Add Biotin-Conjugated anti-monkey IL-2 antibody to appropriate wells. Incubate the plate.



Wash and add prepared Streptavidin-HRP Conjugate to appropriate wells. Incubate at room temperature.



Wash and add TMB Substrate to each well. Incubate before addition of Stop Solution to each well. Read immediately.

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. Modifications to the kit components or procedures may result in loss of performance.

4. STORAGE AND STABILITY

Store kit at 2-8ºC immediately upon receipt.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in section 9 Reagent Preparation.

5. MATERIALS SUPPLIED

Item	Amount	Storage Condition (Before Preparation)
Microplate coated with monoclonal antibody to monkey IL-2	96 wells	2-8 ºC
Biotin-Conjugate anti-monkey IL-2 polyclonal antibody	70 µL	2-8 ºC
Streptavidin-HRP	150 µL	2-8 ºC
Monkey IL-2 Standard lyophilized	2 Vials	2-8 ºC
20X Assay Buffer	5 mL	2-8 ºC
20X Wash Buffer	50 mL	2-8 ºC
Sample Diluent	12mL	2-8 ºC
TMB Substrate Solution	15 mL	2-8 ºC
Stop Solution	15 mL	2-8 ºC

6. MATERIALS REQUIRED, NOT SUPPLIED

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

- 5 mL and 10 mL graduated pipettes
- 5 µL to 1000 µL adjustable single channel micropipettes with disposable tips
- 50 µL to 300 µL adjustable multichannel micropipette with disposable tips
- Multichannel micropipette reservoir
- Beakers, flasks, cylinders necessary for preparation of reagents
- Device for delivery of wash solution (multichannel wash bottle or automatic wash system)
- Microplate strip reader capable of reading at 450 nm (620 nm as optional reference wave length)
- Glass-distilled or deionized water
- Statistical calculator with program to perform regression analysis

7. LIMITATIONS

- Assay kit intended for research use only. Not for use in diagnostic procedures
- Do not use kit or components if it has exceeded the expiration date on the kit labels
- 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

8. TECHNICAL HINTS

- 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.
- As exact conditions may vary from assay to assay, a standard curve must be established for every run.
- Disposable pipette tips, flasks or glassware are preferred, reusable glassware must be washed and thoroughly rinsed of all detergents before use.
- Improper or insufficient washing at any stage of the procedure will result in either false positive or false negative results. Empty wells completely before dispensing fresh wash solution, fill with Wash Buffer as indicated for each wash cycle and do not allow wells to sit uncovered or dry for extended periods.
- **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.

9. REAGENT PREPARATION

Equilibrate all reagents and samples to room temperature (18-25°C) prior to use. If crystals have formed in the Buffer Concentrates, warm them gently until they have completely dissolved.

9.1 1X Wash Buffer

Prepare 1X Wash Buffer by diluting the 20X Wash Buffer Concentrate with distilled or deionized water. To make 500 mL 1X Wash Buffer, combine 25 mL 20X Wash Buffer Concentrate with 475 mL distilled or deionized water. Mix thoroughly and gently to avoid foaming.

Note: The 1X Wash Buffer should be stored at 2-8 ºC and is stable for 30 days.

9.2 1X Assay Buffer

Prepare 1X Assay Buffer by diluting the 20X Assay Buffer Concentrate with distilled or deionized water. To make 50 mL 1X Assay Buffer, combine 2.5 mL 20X Assay Buffer Concentrate with 47.5 mL distilled or deionized water. Mix thoroughly and gently to avoid foaming.

Note: The 1X Assay Buffer should be stored at 2-8 ºC and is stable for 30 days.

9.3 1X Biotin Conjugated Antibody

To prepare the Biotin Conjugated Antibody, dilute the anti-monkey IL-2 polyclonal antibody 100-fold with 1X Assay Buffer. Use the following table as a guide to prepare as much 1X Biotin Conjugated Antibody as needed by adding the required volume (µL) of the Biotin Conjugated Antibody to the required volume (mL) of Assay Buffer. Mix gently and thoroughly.

Number of strips	Volume of Biotin-Conjugated IL-2 antibody (µL)	1X Assay Buffer (mL)
1 - 6	30	2.97
7 - 12	60	5.94

Note: The 1X Biotin-Conjugated Antibody should be used within 30 minutes after dilution.

9.4 1X Streptavidin-HRP Conjugate

To prepare the Streptavidin-HRP Conjugate, dilute the anti-Streptavidin-HRP Conjugate 100-fold with 1X Assay Buffer. Use the following table as a guide to prepare as much 1X Streptavidin-HRP Conjugate as needed by adding the required volume (µL) of the Streptavidin-HRP Conjugate to the required volume (mL) of distilled water. Mix gently and thoroughly.

Number of strips	Volume of Streptavidin-HRP (µL)	1X Assay Buffer (mL)
1 - 6	60	5.94
7 - 12	120	11.88

Note: The 1X Streptavidin-HRP should be used within 30 minutes after dilution.

- All other solutions are supplied ready to use

10. STANDARD PREPARATIONS

Prepare serially diluted standards immediately prior to use. Always prepare a fresh set of standards for every use.

10.1 Prepare a 2400 U/mL **Stock Standard** by reconstituting the Monkey IL-2 standard with the volume of distilled water stated on the label. Hold at room temperature for 10-30 minutes. The 2400 U/mL **Stock Standard** cannot be stored for later use.

10.2 Label eight tubes with numbers 1 - 8.

10.3 Add 225 µL Sample diluent into all tubes.

10.4 Prepare a 1200 U/mL **Standard 1** by adding 225 µL of the 2400 U/mL Stock Standard to 225 µL sample diluent to tube 1. Mix thoroughly and gently.

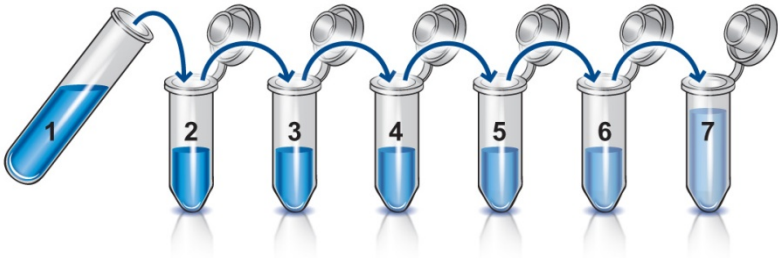
10.5 Prepare **Standard 2** by transferring 225 µL from Standard 1 to tube 2. Mix thoroughly and gently.

10.6 Prepare **Standard 3** by transferring 225 µL from Standard 2 to tube 3. Mix thoroughly and gently.

10.7 Using the table below as a guide, repeat for tube numbers 4 through to 7.

10.8 **Standard 8** contains no protein and is the Blank control

Standard	Sample to Dilute	Volume to Dilute (µL)	Volume of Diluent (µL)	Starting Conc. (U/mL)	Final Conc. (U/mL)
1	Stock	225	225	2400.0	1200.0
2	Standard 1	225	225	1200.0	600.0
3	Standard 2	225	225	600.0	300.0
4	Standard 3	225	225	300.0	150.0
5	Standard 4	225	225	150.0	75.0
6	Standard 5	225	225	75.0	37.5
7	Standard 6	225	225	37.5	18.8
8	None	-		-	-



11. SAMPLE COLLECTION AND STORAGE

- Cell culture supernatant, serum and plasma (EDTA, citrate, heparin), amniotic fluid, urine and whole blood were tested with this assay. Other biological samples might be suitable for use in the assay. Remove serum from the clot or cells as soon as possible after clotting and separation.

- Samples containing a visible precipitate must be clarified prior to use in the assay. Do not use grossly hemolyzed or lipemic specimens.

- Possible “Hook Effects” may be observed due to high sample concentrations. It is recommended to run several dilutions of your sample to ensure an accurate reading.

- Samples should be aliquoted and must be stored frozen at -20°C to avoid loss of bioactive Monkey IL-2. If samples are to be run within 24 hours, they may be stored at 2° to 8°C.

- Avoid repeated freeze-thaw cycles. Prior to assay, the frozen sample should be brought to room temperature slowly and mixed gently and properly diluted with 1X Sample Diluent.

- Aliquots of serum and cell culture supernatant samples (spiked or unspiked) were stored at -20°C and thawed 5 times, and the monkey IL-2 levels determined. There was no significant loss of monkey IL-2 immunoreactivity detected up to 3 cycles of freezing and thawing. A significant decrease of monkey IL-2 immunoreactivity (20%) was detected at further freeze-thaw cycles.

- Aliquots of serum and cell culture supernatant samples (spiked or unspiked) were stored at -20°C, 2-8°C, room temperature (RT) and at 37°C, and the monkey IL-2 level determined after 24 h. There was no significant loss of monkey IL-2 immunoreactivity detected during storage at -20°C, 2-8°C and RT. A significant loss of monkey IL-2 immunoreactivity (20%) was detected during storage at 37°C after 24 h.

12. PLATE PREPARATION

- The 96 well plate strips included with this kit are supplied ready to use.
- Unused well strips should be returned to the plate packet and stored at 2-8°C
- For statistical reasons, we recommend each sample should be assayed with a minimum of two replicates (duplicates)
- Well effects have not been observed with this assay.

13. ASSAY PROCEDURE

- **Equilibrate all materials and prepared reagents to room temperature prior to use.**
- **It is recommended to assay all standards, controls and samples in duplicate.**

13.1. 2 x 50 µL samples are needed for duplicate measurement. Serum or plasma samples are applied undiluted.

13.2. Monkey IL-2 levels in cell culture supernatants may vary considerably. Optimal dilution has to be determined for each individual sample. For unknown cell culture samples it is useful to analyze undiluted as well as prediluted samples (e.g. 1:20 - 1:50) in parallel, thereby covering a wider range in one assay. Cell culture supernatants with very high concentrations of Monkey IL-2 require high dilutions (e.g. up to 1:2000) in order to be measured correctly. Such samples must be prediluted in the respective cell culture medium. Final dilution has to be performed in Sample Diluent according to the following scheme:

Dilution	Sample Volume	Sample Diluent	Dilution Factor
1 : 5	50 µL	200 µL	5
1 : 10	25 µL	225 µL	10
1 : 50	10 µL	490 µL	50
1 : 100	A: 10 µL Sample B: 25 µL predilution A	90 µL 225 µL	100
1 : 1000	A: 10 µL Sample B: 25 µL predilution A	990 µL Culture Medium 225 µL	1000
1 : 2000	A: 10 µL Sample B: 10 µL predilution A	390 µL Culture Medium 490 µL	2000

13.3. Prepare all reagents, working standards, and samples as directed in the previous sections. Determine the number of microplate strips required to test the desired number of

samples plus appropriate number of wells needed for running blanks and standards.

13.4. Wash the microplate twice with approximately 400 µL 1X Wash Buffer per well with thorough aspiration of microplate contents between washes. Allow the 1X Wash Buffer to remain in the wells for about 10 - 15 seconds before aspiration. Take care not to scratch the surface of the microplate.

13.5. After the last wash step, empty wells and tap microplate on absorbent pad or paper towel to remove excess 1X Wash Buffer. Use the microplate strips immediately after washing. Alternatively the microplate strips can be placed upside down on a wet absorbent paper for not longer than 15 minutes. Do not allow wells to dry.

13.6. Pipette 100 µL of each standard dilution into appropriate wells, including the no protein control.

13.7. Add 100 µL of 1X Sample Diluent in duplicate to the blank wells.

13.8. Add 50 µL of 1X Sample Diluent to all sample wells.

13.9. Add 50 µL of each sample in duplicate to the sample wells.

13.10. Add 50 µL of 1X Biotin-Conjugated Antibody to all wells.

13.11. Cover with adhesive film and incubate at room temperature (18° to 25°C) for 2 hours (microplate can be incubated on a shaker set at 400 rpm).

13.12. Remove adhesive film and empty wells. Wash microplate strips 6 times according to step 13.4. Proceed immediately to step 13.12.

13.13. Add 100 µL of Streptavidin-HRP to all wells, including the blank wells.

13.14. Cover with adhesive film and incubate at room temperature (18° to 25°C) for 1 hour (microplate can be incubated on a shaker set at 400 rpm).

13.15. Remove adhesive film and empty wells. Wash microplate strips 6 times according to step 13.4. Proceed immediately to step 13.15.

13.16. Add 100 µL of TMB Substrate Solution to all wells.

13.17. Incubate the microplate strips at room temperature (18 to 25°C) for 30 minutes. Avoid direct exposure to intense light.

Note: The color development on the plate should be monitored and the substrate reaction stopped (see step 13.17) before the signal in the positive wells becomes saturated. Determination of the ideal time period for color development should to be done individually for each assay. It is recommended to add the stop solution when the highest standard has developed a dark blue color. Alternatively the color development can be monitored by the ELISA reader at 620 nm. The substrate reaction should be stopped as soon as Standard 1 has reached an OD of 0.9 - 0.95.

13.18. Stop the enzyme reaction by adding 100 µL of Stop Solution into each well.

Note: It is important that the Stop Solution is mixed quickly and uniformly throughout the microplate to completely inactivate the enzyme. Results must be read immediately after the Stop Solution is added or within one hour if the microplate strips are stored at 2 - 8°C in the dark.

13.19. Read absorbance of each microplate on a spectrophotometer using 450 nm as the primary wave length (optionally 620 nm as the reference wave length; 610 nm to 650 nm is acceptable). Blank the plate reader according to the manufacturer's instructions by using the blank wells. Determine the absorbance of both the samples and the standards.

14. CALCULATIONS

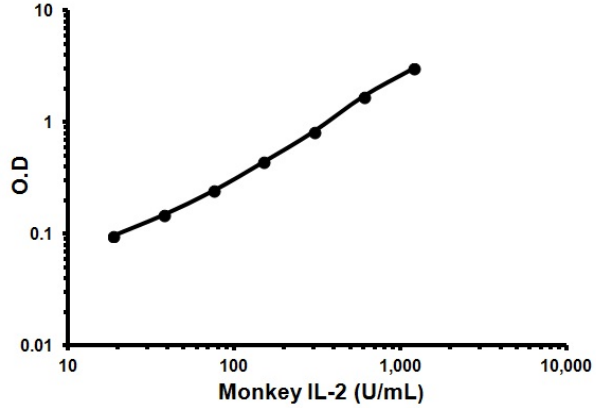
Average the duplicate reading for each standard, sample and control blank. Subtract the control blank from all mean readings. Plot the mean standard readings against their concentrations and draw the best smooth curve through these points to construct a standard curve. Most plate reader software or graphing software can plot these values and curve fit. A five parameter algorithm (5PL) usually provides the best fit, though other equations can be examined to see which provides the most accurate (e.g. linear, semi-log, log/log, 5-parameter logistic). Extrapolate protein concentrations for unknown and control samples from the standard curve plotted. Samples producing signals greater than that of the highest standard should be further diluted in appropriate buffer and reanalyzed, then multiplying the concentration found by the appropriate dilution factor.

If instructions in this protocol have been followed cell culture samples have been diluted. The concentration read from the standard curve must be multiplied by the dilution factor.

Calculation of samples with a concentration exceeding standard 1 may result in incorrect, low monkey IL-2 levels (Hook Effect). Such samples require further external predilution according to expected monkey IL-2 values with Sample Diluent in order to precisely quantitate the actual monkey IL-2 level.

15. TYPICAL DATA

TYPICAL STANDARD CURVE – Data provided for **demonstration purposes only**. A new standard curve must be generated for each assay performed.



Standard Curve Measurements			
Conc.	**O.D. 450 nm**		**Mean**
(U/mL)	**1**	**2**	**O.D.**
0.0	0.053	0.055	0.054
18.8	0.098	0.095	0.096
37.5	0.150	0.144	0.147
75.0	0.243	0.241	0.242
150.0	0.444	0.430	0.437
300.0	0.836	0.795	0.815
600.0	1.683	1.706	1.694
1,200.0	2.996	3.020	3.008

Figure 1. Example of a Monkey IL-2 protein standard curve.

16. TYPICAL SAMPLE VALUES

EXPECTED VALUES –

There were no detectable monkey IL-2 levels found in healthy donors.

Elevated monkey IL-2 levels depend on the type of immunological disorder.

SENSITIVITY –

The limit of detection for IL-2 defined as the analyte concentration resulting in an absorption significantly higher than the absorption of the dilution medium (mean plus two standard deviations) was determined to be 9.1 U/mL (mean of 6 independent assays).

RECOVERY –

The spike recovery was evaluated by spiking 3 levels of Monkey IL-2 into pooled normal Monkey serum and citrate plasma samples. Recoveries were determined in 2 independent experiments with 4 replicates each. The Unspiked serum and plasma was used as blank in these experiments.

Sample Matrix	Spike High (%)		Spike Medium (%)		Spike Low (%)	
	Mean (%)	Range (%)	Mean (%)	Range (%)	Mean (%)	Range (%)
Serum	94	80 - 111	102	95 – 114	105	91 - 118
Plasma (EDTA)	103	93 – 110	94	86 – 106	97	73 – 111
Plasma (citrate)	117	114 – 118	101	95 – 110	98	82 – 115
Plasma (heparin)	115	110 – 119	102	91 – 113	93	73 – 105
Cell culture supernatant	110	106 - 116	109	98 - 120	108	95 -121

PRECISION –

Intra- and Inter-assay reproducibility was determined by measuring samples containing different concentrations of Monkey IL-2.

	Intra-Assay	Inter-Assay
n=	8	8
%CV	7.0	5.0

DILUTION PARALLELISM –

Serum, plasma, and cell culture supernatant samples with different levels of Monkey IL-2 were analyzed at serial 2 fold dilutions with 4 replicates each. For recovery data see table below.

Sample Matrix	Recovery of Exp. Value		
	Dilution	Range (%)	Mean (%)
Serum	1:4	82 – 95	89
	1:8	80 – 90	84
	1:16	73 – 85	80
Plasma (EDTA)	1:4	92 – 102	97
	1:8	82 - 104	92
	1:16	97 – 122	98
Plasma (citrate)	1:4	87 – 98	93
	1:8	83 – 99	89
	1:16	83 -103	91
Plasma (heparin)	1:4	89 – 96	92
	1:8	81 – 95	90
	1:16	82 – 109	94
Cell culture supernatant	1:4	94 – 99	96
	1:8	81 – 90	86
	1:16	82 - 90	86

17. ASSAY SPECIFICITY

The cross reactivity and interference of circulating factors of the immune system was evaluated by spiking these proteins at physiologically relevant concentrations into a monkey IL-2 positive serum. No cross reactivity or interference was detected.

18. TROUBLESHOOTING

Problem	Cause	Solution
Poor standard curve	Inaccurate pipetting	Check pipettes
	Improper standards 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; change to overnight standard/sample incubation
	Inadequate reagent volumes or improper dilution	Check pipettes and ensure correct preparation
Samples give higher value than the highest standard	Starting sample concentration is too high.	Dilute the specimens and repeat the assay
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 kit	Store the all components as directed.

19. NOTES

For all technical and commercial enquires please go to:
www.abcam.com/contactus
www.abcam.cn/contactus (China)
www.abcam.co.jp/contactus (Japan)