RES10-EN.01

resDetect™ Human Interleukin-3 (IL-3) ELISA Kit (Residue Testing)

Catalog Number：RES-A010

Pack Size: 96 tests

IMPORTANT: Please carefully read this manual before performing your experiment.

For Research Use Only. Not For Use in Diagnostic or Therapeutic Procedure

INTENDED USE

resDetect™ Human Interleukin-3 (IL-3) ELISA Kit (Residue Testing) was developed for the detection and quantitative determination of GMP human IL-3 in cell culture supernates.

It is intended for research use only (RUO).

BACKGROUND

Interleukin 3 is also known as IL-3, is a protein that in humans is encoded by the IL3 gene. Interleukin-3 (IL-3) is an interleukin, a type of biological signal (cytokine) that can improve the body's natural response to disease as part of the immune system. It acts by binding to the interleukin-3 receptor.

To support the development of CAR-T drugs, ACROBiosystems developed Human Interleukin-3 (IL-3) ELISA Kit (Residue Testing) with rigorous methodological validation, which is used evaluation the quality of CAR-T products in drug development and CMC quality control stages. Besides, this kit can also be used for the quantitative determination of GMP human IL-3 Protein (ACROBiosystems, Cat#GMP-L03H18) concentrations.

PRINCIPLE OF THE ASSAY

This assay kit is used to measure the levels of human IL-3 by employing a standard sandwich-ELISA format. The micro-plate in the kit has been pre-coated with Anti-IL-3 Antibody. Firstly, add the standard samples provided in kit and your samples to the plate, incubate and wash the wells. Then add the Biotin-Anti-IL-3 Antibody to the plate and form Antibody-antigen-biotinylated antibody complex, incubate and wash the wells. Next add Streptavidin-HRP to the plate, incubate and wash the wells. At last, load the substrate into the wells and monitor solution color from blue to yellow. The reaction is stopped by the addition of a stop solution and the intensity of the absorbance can be measured at 450 nm and 630 nm. The OD Value reflects the amount of human IL-3 bound.

PRECAUTIONS

1. This kit is for research use only and is not for use in diagnostic or therapeutic applications.
2. The kit is suitable for cell supernatants, serum and plasma samples.

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3. Do not use reagents past their expiration date.

4. Do not mix or substitute reagents with those from other kits or other lot number kits.

5. If samples generate values higher than the highest standard, dilute the samples with the appropriate calibrator diluent and repeat the assay. If cell supernatant samples need step dilution, except for the final dilution with diluent, other intermediate dilutions can be in cell culture medium.

6. Differences in test results can be caused by a variety of factors, including laboratory operator, pipette usage, plate washing technique, reaction time or temperature, and kit storage.

7. This kit is designed to remove or reduce some endogenous interference factors in biological samples, and not all possible influencing factors have been removed.

MATERIALS PROVIDED

Table1. Materials provided

Catalog	Components	Size (96 tests)	Format	Storage	
				Unopened	Opened
RES010-C01	Pre-coated Anti-IL-3 Antibody Microplate	1 plate	Solid	2-8°C	2-8°C
RES010-C02	Human IL-3 Standard	20 μg	Powder	2-8°C	-70°C
RES010-C03	Biotin-Anti-IL-3 Antibody	20 μg	Powder	2-8°C	-70°C
RES010-C04	Streptavidin-HRP	50 μL	Liquid	2-8°C, avoid light	2-8°C, avoid light
RES010-C05	10×Washing Buffer	50 mL	Liquid	2-8°C	2-8°C
RES010-C06	2×Dilution Buffer	50 mL	Liquid	2-8°C	2-8°C
RES010-C07	Substrate Solution	12 mL	Liquid	2-8°C, avoid light	2-8°C, avoid light
RES010-C08	Stop Solution	7 mL	Liquid	2-8°C	2-8°C

Note: *It is recommended that Streptavidin-HRP be centrifuged briefly before use to deposit liquid from the tube wall or cap to the bottom of the tube.*

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SRORAGE

1. Unopened kit should be stored at 2℃-8℃ upon receiving.

2. Find the expiration date on the outside packaging and do not use reagents past their expiration date.

3. The opened kit should be stored per components table. The shelf life is 30 days from the date of opening.

REAGENTS/EQUIPMENT NEEDED BUT NOT SUPPLIED

Single or multi-channel micropipettes and pipette tips: need to meet 10 μL, 300 μL, 1000 μL injection requirements;

37ºC Incubator;

Single or dual wavelength microplate reader with 450 nm and 630 nm filter;

Tubes: 1.5mL,10mL;

Timer;

Reagent bottle;

Deionized or distilled water.

REAGENT PREPARATION

Bring all reagents and samples to room temperature (20℃-25℃) before use. If crystals have formed in buffer solution, place the sample in an 37℃ incubator until the crystals have completely dissolved and bring the solution back to room temperature before use.

According to Table 2, prepare the provided lyophilized product into a storage solution with ultrapure water, dissolve at room temperature for 15 to 30 minutes, and mix by gently pipetting, avoiding vigorous shaking or vertexing. The reconstituted storage solution should be stored at -70°C. It is recommended that the number of freezing and thawing should not exceed 1 time, the size of the aliquot should not be less than 5 μg.

Note: *Considering inevitable minor quantitation variations between protein batches, it is also reasonable to generate the standard curve with specific lot of proteins used for current production for even better accuracy.*

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Table 2. Preparation method

ID	Components	Size (96 T)	Storage solution concentration.	Reconstituted water Vol.
RES010-C02	Human IL-3 Standard	20 μg	200 μg/mL	100 μL
RES010-C03	Biotin-Anti-IL-3 Antibody	20 μg	200 μg/mL	100 μL

RECOMMENDED SAMPLE PREPARATION

1. Working Solution Preparation

1.1 Preparation of 1×Washing Buffer:

Dilute 50 mL 10×Washing Buffer with ultrapure water/deionized water to 500 mL.

1.2 Preparation of 1×Dilution Buffer:

Dilute 50 mL 2×Dilution Buffer with 1×Washing Buffer to 100 mL.

1.3 Preparation of Biotin-Anti-IL-3 Antibody working fluid:

Dilute Biotin-Anti-IL-3 Antibody to 0.05 μg/mL with 1×Dilution Buffer. Please prepare it for one-time use only.

1.4 Preparation of Streptavidin-HRP working fluid:

Dilute Streptavidin-HRP at 1:2000 with 1×Dilution Buffer. The prepared working fluid should avoid light. Please prepare it for one-time use only.

1.5 Sample preparation

a. If the sample to be tested is the cell supernatant, dilute test sample at 1:5 with 1×Dilution Buffer. The volume ratio of sample to diluent is 1:4.

b. If the sample to be tested is serum or plasma, dilute test sample at 1:5 with 1×Dilution Buffer. The volume ratio of sample to diluent is 1:4.

2. Preparation of Standard curve

The concentration of the reconstituted human IL-3 Calibrator (RES010-C02) is 200 μg/mL，prepare (Std.-0) by diluting 10 μL the reconstituted human IL-3 Calibrator into 990 μL Sample Dilution Buffer, mix gently well. Then prepare Std.- 1' by diluting 10 μL Std.-0 into 990 μL Sample Dilution Buffer. At last,

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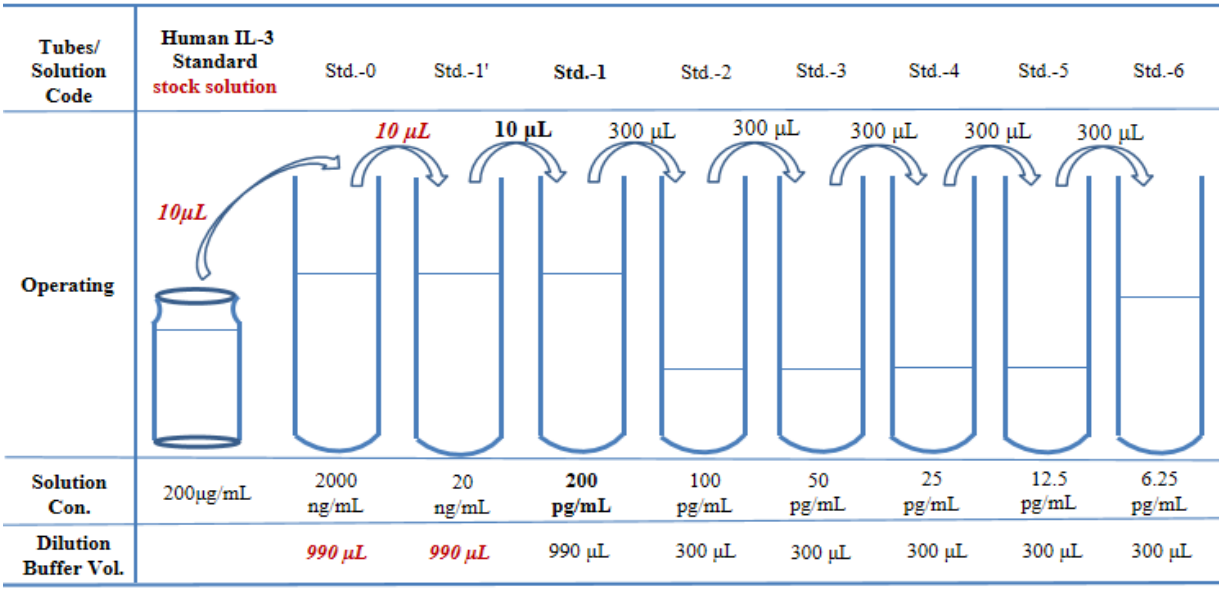
prepare the highest concentration of standard curve, Std.-1 (200 pg/mL), by diluting 10 µL Std.- 1' into 990 µL Sample Dilution Buffer. Prepare 1:1 serial dilution for the standard curve as follows: Pipette 300 µL of Sample Dilution Buffer into each tube. Make sure to mix well every time. Sample Dilution Buffer serves as blank.



3. Add Samples

Add 100 µL Calibrator and samples to each well. For blank Control wells, please add 100 µL Dilution Buffer.

***Note**: It is recommended to set double holes for samples and standard curves to be tested.*

4. Incubation

Seal the plate with microplate sealing film and incubate at room temperature for 1.0 hour.

5. Washing

Remove the remaining solution by aspiration, add 300 µL of 1×Washing Buffer to each well, soak for 10 s, remove any remaining 1×Washing Buffer: by aspirating or decanting, invert the plate and blot it against paper towels. Repeat the wash step above for three times.

6. Add Biotin-Anti-IL-3 Antibody

For all wells, add 100 µL Biotin-Anti-IL-3 Antibody (dilute to 0.05 µg/mL) working solution. Please

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prepare it for one-time use only.

7. Incubation

Seal the plate with microplate sealing film and incubate at room temperature for 1.0 hour.

8. Washing

Repeat step 5.

9. Add Streptavidin-HRP

For all wells, add 100 µL Streptavidin-HRP (dilute at 1:2000) working solution. Please prepare it for one-time use only, avoid light.

10. Incubation

Seal the plate with microplate sealing film and incubate at room temperature for 30 min.

11. Washing

Repeat step 5.

12. Substrate Reaction

Add 100 µL Substrate Solution to each well. Seal the plate with microplate sealing film and incubate at room temperature for 20 min, avoid light.

13. Termination

Add 50 µL Stop Solution to each well and tap the plate gently to allow thorough mixing.

Note: *The color in the wells should change from blue to yellow.*

14. Data Recording

Read the absorbance at 450 nm and 630 nm using UV/Vis microplate spectrophotometer within 10 minutes.

Note*: To reduce the background noise, subtract the value read at* OD_{450nm} *with the value read at* $OD_{630\ nm}$.

CALCULATION OF RESULTS

1. Calculate the mean absorbance for each standard, control and sample and subtract average zero standard optical density (OD).

2. The standard curve is plotted with the standard concentration as x-axis and the calibrated absorbance value as y-axis. Four parameters logistic are used to draw the standard curve and calculate the sample

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concentration.

3. Normal range of Standard curve: $R^2 \geq 0.9900$.

4. Detection range: 6.25 pg/mL-200 pg/mL. If the OD value of the sample to be tested is higher than 200 pg/mL, the sample shall be diluted with dilution buffer and assay repeated. If the OD value of the sample to be tested is lower than 6.25 pg/mL, the sample should be reported.

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QUICK GUILD

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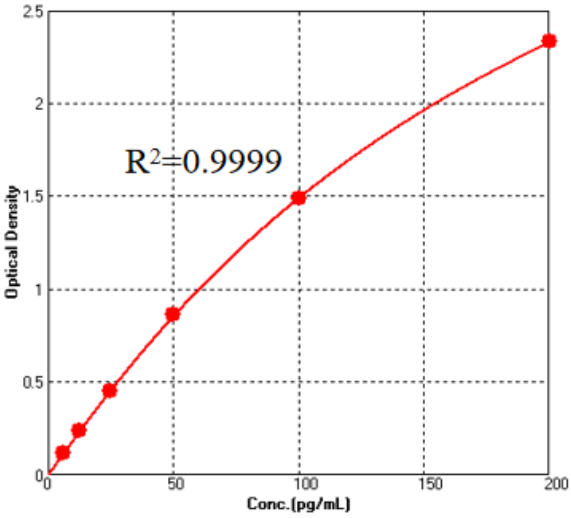
TYPICAL DATA

For each experiment, a standard curve needs to be set for each micro-plate, and the specific OD value may vary depending on different laboratories, testers, or equipments. The following example data is for reference only. The sample concentration was calculated based on the results of the standard curve.

Standard (pg/mL)	O.D.-1	O.D.-2	Average	Corrected
200	2.372	2.365	2.369	2.330
100	1.519	1.538	1.529	1.490
50	0.902	0.904	0.903	0.864
25	0.491	0.484	0.488	0.449
12.5	0.276	0.277	0.277	0.238
6.25	0.158	0.156	0.157	0.118
0	0.038	0.040	0.039	/



SENSITIVITY

The minimum detectable concentration of IL-3 is 2.590 pg/mL. The minimum detectable concentration was determined by adding twice standard deviations to the mean optical density value of twenty zero standard replicates and calculating the corresponding concentration.

PRECISION

1. Intra-assay Precision

Three samples of known concentration were tested ten times on one plate to assess intra-assay precision.

2. Inter-assay Precision

Three samples of known concentration were tested in three separate assays to assess inter-assay precision.

	Intra-assay Precision			Inter-assay Precision		
Sample	1	2	3	1	2	3
n	10	10	10	3	3	3
Mean (pg/mL)	155.413	39.269	15.203	154.111	39.556	15.137

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SD	4.016	0.966	0.479	3.518	0.370	0.358
CV (%)	2.6	2.5	3.1	2.3	0.9	2.4

***Note**: The example data is for reference only.*

RECOVERY

Five parts of blank T cell culture supernatant were added with different concentrations of human IL-3, and the T cell culture supernatant without human IL-3 was used as background to calculate the recovery rate. The range of recovery rate is 92.1-97.4%, and the average recovery is 93.8%.

Sample Type	Average % Recovery	Range
T cell culture supernatant (n=5)	93.8	92.1-97.4

LINEARITY

To assess the linearity of the assay, samples spiked with high concentrations of IL-3 were serially diluted with calibrator diluent to produce samples with values within the dynamic range of the assay.

		Cell culture medium (DMEM)	Cell culture medium (1640)	Serum
1:2	Average Recovery (%)	90.6	90.3	92.4
	Range (%)	87.0-94.2	87.0-94.4	90.1-93.5
1:4	Average Recovery (%)	89.8	96.7	88.3
	Range (%)	83.9-94.4	93.8-102.2	84.0-90.7
1:8	Average Recovery (%)	90.3	85.5	91.2
	Range (%)	89.0-91.2	83.7-86.4	89.2-92.6
1:16	Average Recovery (%)	89.8	89.9	93.0
	Range (%)	87.5-92.9	87.8-93.5	88.7-98.0

***Note**: The example data is for reference only.*

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SPECIFICITY

This assay recognizes natural and recombinant human IL-3. No cross-reactivity was observed when this kit was used to analyze the following recombinant cytokines.

Human			
IL-2	IL-12B	SCF	M-CSF
IL-4	IL-15	Flt-3 Ligand	GM-CSF
IL-5	IL-17A	Thrombopoietin-TPO	Anti-CD3
IL-6	IL-18	TGF-beta 1	Anti-CD28
IL-7	TNF-alpha	VEGF165	Anti-CD137
IL-8	IFN-gamma	L1R	
IL-10	IFN-alpha 1	BMP-2	
IL-11	FGF basic	G-CSF	

INTERFERING SUBSTANCES

Verify potential matrix effects by adding different levels of DMSO and HSA to the diluted buffer.

Additive	Tolerated concentration
DMSO	10%
HSA	5%

CALIBRATION

This immunoassay is calibrated against a highly purified *E. coli*-expressed recombinant human IL-3(91/510). Reference Reagent is calibrated by NIBSC/WHO in April 2013.

NIBSC/WHO (91/510) approximate value (U/mL) = 0.003 × Human IL-3 value (pg/mL).

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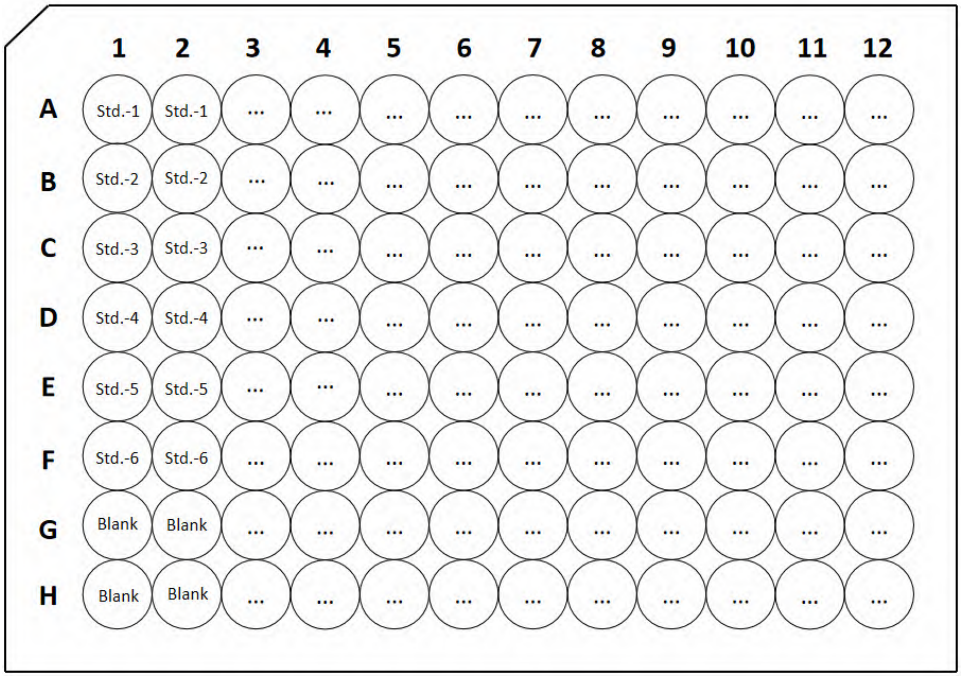
PLATE LAYOUT



Note: *Blank is a Blank Dilution Buffer hole.*

TROUBLESHOOTING GUIDE

Problem	Cause	Solution
Poor standard curve	* Inaccurate pipetting	* Check pipettes
Large CV	* Inaccurate pipetting * Air bubbles in wells	* Check pipettes * Remove bubbles in wells
High background	* Plate is insufficiently washed * Contaminated wash buffer	* Review the manual for proper wash. * Make fresh wash buffer
Very low readings across the plate	* Incorrect wavelengths * Insufficient development time	* Check filters/reader * Increase development time

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Samples are reading too high, but standard curve looks fine	* Samples contain cytokine levels above assay range	* Dilute samples and run again
Drift	* Interrupted assay set-up * Reagents not at room temperature	* Assay set-up should be continuous - have all standards and samples prepared appropriately before commencement of theassay * Ensure that all reagents are at room temperature before pipetting into the wells unless otherwise instructed in the antibody inserts

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