Primacu[™] ELISA

Human Interleukin-17 receptor A ELISA Kit

User instruction

Cat. No. BPE151

Standard Curve Range: 31.25-2000 pg/mL

Sensitivity: 6.4 pg/mL

Size: 5*96T, 96T, 15*96T

*This product is for research use only, not for use in diagnosis procedures. It's highly recommended to read this instruction entirely before use.

Precision

Intra-Assay Precision (Precision within an assay) Three samples of known concentration were tested on one plate to assess intra-assay precision.

Inter-Assay Precision (Precision between assays) Three samples of known concentration were tested in separate assays to assess inter-assay precision. CV(%) = SD/mean x 100

Intra/Inter-Assay Precision	Sample	n	Mean	Standard Deviation	CV%
Intra-assay	1	20	133	6.9	5.2
Intra-assay	2	20	428	15.7	3.7
Intra-assay	3	20	1188	36.6	3.1
Inter-assay	1	20	133	11.6	8.7
Inter-assay	2	20	426	41.4	9.7
Inter-assay	3	20	1201	117	9.8

Intended Us

This sandwich kit is for the accurate quantitative detection of Human Interleukin-17 receptor A (also known as IL17RA) in serum, plasma, cell culture supernatant. The other biological fluids need to be validated by the end user due to the complexity of natural targets and unpredictable interference.

Assav Principle

This is an Enzyme-Linked Immunosorbent Assay (ELISA) kit designed to detect Human IL17RA. The assay plate has been pre-coated with mouse anti-Human IL17RA monoclonal antibody. When the sample containing IL17RA is added to the plate, it binds to the antibodies coated on the wells. Then, a horseradish peroxidase conjugated mouse anti-Human IL17RA Antibody is added to the wells and binds to IL17RA in the sample. After washing the wells, substrate solutions are added, and the color intensity is directly proportional to the amount of Human IL17RA present. The reaction is stopped by adding an acidic stop solution, and the absorbance is measured at 450 nm.

Reagents Provided

Components	Specifications	Storage condition	
Pre-coated ELISA Plate	12 × 8 well strips × 1	Return unused wells to the foil pouch containing the desiccant pack and store for up to 1 month at 2-8°C .	
Standard Solution	0.2mL × 1 vail	Store at -20°C in a manual defrost freezer until expiration date. Avoid repeated freeze-thaw cycles. Reconstituted working solution should be freshly prepared and discard after use.	
Dilution Buffer	20mL × 1 vail		
Stop Solution	6mL × 1 vail		
Substrate Solution A	12mL × 1 vail		
Substrate Solution B	12mL × 1 vail	Unopened reagents can store at 2-8°C until expiration date.Store opened reagents at 2-8°C and use within one month.	
Wash Buffer Concentrate (25x)	20mL × 1 vail		
Detection Antibody Concentrate	0.6mL × 1 vail		
User manual	1		
Plate Sealers	2pcs		
Zipper bag	1pcs		

Material Required But Not Supplied

- Absorbent paper Precision pipettes and disposable pipette tips
- Clean tubes Deionized or distilled water
- Microplate reader with 450 ± 10nm wavelength filter

Precautions

- This instruction must be strictly followed in the experiment. Store kit at 2 8°C. Do not use reagents after expiration date.
- All reagent bottle caps must be tightened to prevent evaporation and microbial pollution.
- Substrate Solution B is sensitive to ignore the keyses Substrate Solution B to light. Stop solution contains acid. Please wear eye, hand and skin protection when using this material.
- . The kit should not be used beyond the expiration date.

Specimen Collection

Serum Allow serum to clot for 30 minutes at room temperature. Then centrifuge at 1,000 × g for 15 minutes. Collect the supernatant without sediment and assay immediately. Plasma Collect plasma using EDTA or heparin as an anticoagulant. After mix 10-20 minutes, centrifuge samples for 15 minutes at 1,000 × g. Collect the supernatant without sediment and assay

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Cell culture supernatant Remove particulates by centrifugation at 500 × g for 5 minutes. Collect the supernatant without sediment and assay immediately

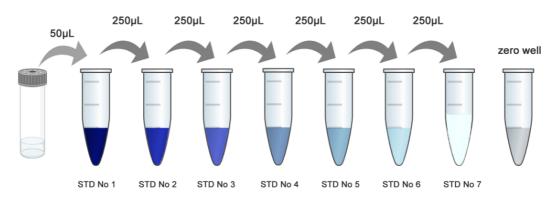
Note

Aliquot and store samples at ≤ -20°C. Avoid repeated freeze-thaw cycles

- Samples should be brought to room temperature before starting the assay.
- Collect the supernatations carefully. When sediments occurred during storage, centrifugation should be performed again. Hemolysis can greatly impact the validity of test results. Take care to minimize hemolysis.
- Reagent Preparation

 All reagents should be brought to room temperature (22 28°C) equilibration (at least 30 minutes) before use.
- Standard Solution Reconstitute 50µL of the standard solution (20 ng/mL) with 450µL of Dilution Buffer to generate a stock standard solution of 2ng/mL (Standard No.7). Allow the standard to sit for 15 minutes with gentle agitation prior to making dilutions. Prepare duplicate standard points by serially diluting the standard stock solution (2ng/mL) 1:2 with Dilution Buffer to produce 1ng/mL, 0.5ng/mL, 0.25ng/mL, 0.125ng/mL, 0.0625ng/mL and 0.03125ng/mL solutions. Dilution Buffer serves as the zero standard (0 pg/µL). Any remaining standard solution should be frozen at -20°C and used for up to one month. Dilution of standard solutions suggested are as follows:

Concentration	Standard No.	Dilution Steps	
2ng/mL	Standard No.1	50µL Original Standard + 450µL Dilution Buffer	
1ng/mL	Standard No.2	250μL Standard No.7 + 250μL Dilution Buffer	
0.5ng/mL	Standard No.3	250μL Standard No.6 + 250μL Dilution Buffer	
0.25ng/mL	Standard No.4	250μL Standard No.5 + 250μL Dilution Buffer	
0.125ng/mL	Standard No.5	250μL Standard No.4 + 250μL Dilution Buffer	
0.0625ng/mL	Standard No.6	250μL Standard No.3 + 250μL Dilution Buffer	
0.03125ng/mL	Standard No.7	250μL Standard No.2 + 250μL Dilution Buffer	
0ng/mL	Standard No.0	250µL Dilution Buffer	



- Wash Buffer Add 20 µL of Wash Buffer Concentrate to 480 µL of deionized or distilled water to prepare 500 µL of 1× Wash Buffer. If crystals have formed in the concentrate, warm to room ٠ temperature and mix gently until the crystals have completely dissolved. Solution Substrate Solution A and B should be mixed together in equal volumes within 10 minutes of use. Protect from light. Each well requires 200 µL of the resultant mixture.
- Detection Antibody Solution Add 0.6 mL of Detection Antibody Concentrate to 11.4 mL Dilution Buffer to prepare 12 mL Detection Antibody Solution

Assay Procedure

- 1. Prepare all reagents, standard solutions, samples and Detection Antibody Solutions as instructed. Bring all reagents to room temperature before use. The assay is performed at room temperature. It is recommended that all standards and samples be assayed in duplicate. Determine the number of strips needed for the assay and insert them into the frames provided. Store unused strips at 2-8°C
- 2. Add 100 µL standard or sample per well. Cover the plate with a sealer and incubate 2 hours at room temperature.
- 3. Discard the contents of the plate. Add 300 µL of 1× Wash Buffer to each well and soak for 1 minute. Then aspirate or decant the liquid and pat the wells dry with clean paper towels. Repeat the process twice for a total of three washes. Ensure complete removal of liquid at each step. Note: Improper washes may lead to falsely elevated signals and poor reproducibility.
- 4. Add 100 μ L of the Detection Antibody Solution to each well. Seal the plate and incubate 1 hour at room temperature.

5. Repeat the aspiration/wash as in Step 3.

- 6. Add 200 µL of Substrate Solution (Pre-mixed in reagent preparation step) to each well. Incubate for 20 minutes at room temperature. Protect from light.
- 7. Add 50 µL Stop Solution to each well to stop the reaction. The solution color in the wells should change from blue to yellow. Note: If color change does not appear uniform, gently tap the plate to ensure thorough mixing
- 8. Determine the optical density (OD value) of each well immediately using a microplate reader set to 450 nm within 10 minutes after adding the stop solution.

Summarv

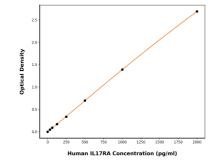
- 1. Prepare all reagents, standard solutions and samples.
- 2. Add standard or samples into each well. Incubate for 2 hours at room temperature.
- 3. Wash the plate 3 times
- 4. Add Detection Antibody Solution into each well. Incubate for 1 hour at room temperature.
- 5. Wash the plate 3 times.
- 6. Add Substrate Solution. Incubate for 20 minutes at room temperature. Protect from light.
- 7. Add Stop Solution and color develops
- 8. Read the OD value within 10 minutes.

Calculation of Result

- 1. If samples generate values higher than the highest standard, dilute the samples and repeat the assay, then the concentration read from the standard curve must be multiplied by the dilution factor
- 2. Calculate the mean absorbance for each standard and sample, and subtract average zero standard optical density.
- 3. The data been calculated by 4-parameter logistics curve-fitting algorithm.

Typical Data

This standard curve is for demonstration purpose only. A standard curve should be generated with each assay



Concentration	OD Value	Average	Corrected	
2000 pg/ml	2.832	2.815	2.689	
2000 þýrin	2.798	2.015		
1000 pg/ml	1.594	1.515	1.389	
	1.436	1.515		
500 pg/ml	0.855	0.823	0.697	
500 þg/m	0.791	0.823		
250 pg/ml	0.471	0.461	0.335	
230 þýrm	0.451	0.401		
125 pg/ml	0.278	0.297	0.171	
125 pg/m	0.316	0.231		
62.5 pg/ml	0.228	0.217	0.091	
62.5 pg/m	0.206	0.217		
31.25 pg/ml	0.164	0.172	0.046	
31.25 pg/m	0.18	0.172		
0 pg/ml	0.121	0.126	0	
0 pg/m	0.131	0.120	, , , , , , , , , , , , , , , , , , ,	

Troubleshooting

Possible Case	Solution				
High Background					
Insufficient washes	 See washing procedure on the user manual Increase number of washes 				
Weak Signal					
Improper washing Incorrect incubation temperature Reagent are contaminated Pipette are not clean	Increasing duration of soaking steps ncubate at room temperature Use new one Pipette should be clean				
No Signal					
 Reagents added in incorrect order, or incorrectly prepared Standard curve looks fine but there is no signal in the sample wells Standard wells no signal but there is a signal in the sample wells 	 Review manual and repeat assay Check if the samples were handled and stored correctly. Check that standard was handled according to manual. 				
Poor Standard Curve					
Plate not developed long enough Incorrect procedure Imprecise / inaccurate pipetting	 Increase Substrate Solution incubation time Review manual and repeat the assay to establish the standard curve. Check and calibrate the pipettes 				

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