Dupilumab ELISA Kit

Cat #: orb2975745 (manual)

For research use only. Not intended for diagnostic use.

Product Features

Intend Use: Used for the quantitative determination of Dupilumab concentration in serum and plasma.

Detectable Sample Type: Serum, Plasma

Detection Range: 78.125 ng/mL - 5, 000 ng/mL

Sensitivity: The minimum detectable dose (MDD) of Dupilumab is typically less than 48.20 ng/mL.

Assay Principle

This assay employs the quantitative competitive enzyme immunoassay technique. Recombinant human CD124 has been pre-coated onto a microplate. Standards or samples are premixed with biotin-labeled antibody and then pipetted into the wells. Dupilumab in the sample competitively binds to the pre-coated protein with biotin-labeled Dupilumab. After washing away any unbound substances, Streptavidin-HRP is added to the wells. Following a wash to remove any unbound enzyme reagent, a substrate solution is added to the wells and color develops in inversely proportion to the amount of Dupilumab bound in the initial step. The color development is stopped, and the intensity of the color is measured.

Kit Components

Item	Size	Description	Storage Conditions
Pre-coated Microplate	1 plate	96 well polystyrene microplate (12 strips of 8 wells) precoated with recombinant human CD124.	Store in sealed at -20°C.
Dupilumab Standard	2 bottles	5,000 ng/bottle of lyophilized Dupilumab. Reconstitute in 1 mL Standard Diluent before used.	Store at -20°C.
Detection A	1 vial	60 µL/vial of Biotin labeled Dupilumab antibody (including preservative), 1:100 diluted by Assay Diluent before used.	Store at -20°C.
Detection B	1 vial	120 µL/vial of Streptavidin-HRP (including preservative), 1:100 diluted by Assay Diluent before used.	Store at -20°C.
Standard Diluent	1 bottle	25 mL/bottle diluent (including preservative) was used to dilute the Standard and Samples.	Store at 4°C.
Assay Diluent	1 bottle	25 mL/bottle diluent (including preservative) was used to dilute the Detection A and Detection B.	Store at 4°C.
20 × Wash Buffer	1 bottle	25 mL/bottle of a 20-fold concentrated solution of buffered surfactant with preservative, 1:20 diluted by deionized water before used.	Store at 4°C.
Color Reagent	1 bottle	12 mL/ bottle of TMB (Tetramethylbenzidine).	Store at 4°C.
Stop Solution	1 bottle	6 mL/ bottle.	Store at 4°C.

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Plate Sealers	4 strips	Adhesive strips.	Store at RT.

Materials Required but Not Supplied

1. Microplate reader capable of measuring absorbance at 450 nm, with the correction wavelength set at 630 nm or 620 nm.
2. Pipettes and pipette tips.
3. Deionized or distilled water.
4. 500 mL graduated cylinder.
5. Squirt bottle, manifold dispenser, or automated microplate washer.
6. Test tubes for dilution of standards.

Sample Preparation

Sample Collection

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Handle all blood and serum as if capable of transmitting infectious agents. The NCCLS provides recommendations for handling and storing serum and plasma specimens (Approved Standard-Procedures for the Handling and Processing of Blood Specimens, H18-A. 1990).

Serum: Use a serum separator tube (SST) and allow samples to clot for 30 minutes at room temperature before centrifugation for 15 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at ≤ -20℃. Avoid repeated freeze-thaw cycles.

Plasma: Collect plasma using EDTA, heparin, or citrate as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20℃. Avoid repeated freeze-thaw cycles.

Reagent Preparation

Bring all reagents to room temperature before use.

20-fold Wash Buffer Concentrate - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Add 25 mL of Wash Buffer Concentrate to 475 mL of deionized or distilled water to prepare 500 mL of Wash Buffer.

Serum and Plasma - Serum and plasma samples require a 10-fold dilution. A suggested 10-fold dilution is 10 µL of sample + 90 µL of Standard Diluent (diluted 1:9). If the sample value is outside the range of the standard curve, the dilution can be adjusted appropriately, and the assay can be redetermined. If the antibody concentration in the sample can be estimated and the assay can be performed simultaneously by diluting several gradients prior to the experiment.

Standard - Reconstitute with 1 mL Standard Diluent, this reconstitution produces a stock solution of 5,000 ng/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions. 5,000 ng/mL is the first standard point, and the concentration of the 7 standard sample were 5,000 ng/mL, 2,500 ng/mL, 1,250 ng/mL, 625 ng/mL, 312.5 ng/mL, 156.25 ng/mL, 78.13 ng/mL respectively. The appropriate Standard Diluent serves as the zero standard (0 ng/mL).

Detection A (working solution) - Shake and mix before used. Centrifuge instantaneously with palm centrifuge to make the liquid at the bottom of the tube. Dilute the Detection A 1: 100 times to the working concentration with Assay Diluent.

Detection B (working solution) - Shake and mix before used. Centrifuge instantaneously with palm centrifuge to make the liquid at the bottom of the tube. Dilute the Detection B 1: 100 times to the working concentration with Assay Diluent.

Assay Procedure

Bring all reagents and samples to room temperature before use. It is recommended that all standards and samples be assayed in duplicate.

1. Prepare all reagents and working standards as directed in the previous sections.
2. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal.
3. Add 100 µL premixed solution (50 µL diluted standard/sample + 50 µL Detection A working solution) to each well. Cover with the adhesive strip provided. Incubate for 2 hours at 37℃.
4. Aspirate each well and wash, repeating the process three times. Wash by filling each well with Wash Buffer (300 µL) using a squirt bottle, manifold dispenser, or autowasher. 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 µL of Detection B (working solution) to each well. Cover with a new adhesive strip. Incubate for 30 minutes at 37℃.
6. Aspirate each well and wash, repeating the process five times. Wash by filling each well with Wash Buffer (300 µL) using a squirt bottle, manifold dispenser, or autowasher. 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.
7. Add 100 µL of Color Reagent to each well. Incubate for 15 minutes at 37℃. Protect from light.
8. Add 50 µL of Stop Solution to each well. The color in the wells should change from blue to yellow. If the color in the wells is green or the color change does not appear uniform, gently tap the plate to ensure thorough mixing.
9. Determine the optical density of each well within 10 minutes, using a microplate reader set to 450 nm. If wavelength correction is available, set to 630 nm or 620 nm. If wavelength correction is not available, subtract readings at 630 nm or 620 nm from the readings at 450 nm. This subtraction will correct for optical imperfections in the plate. Readings made directly at 450 nm without correction may be higher and less accurate.

Calculation of Results

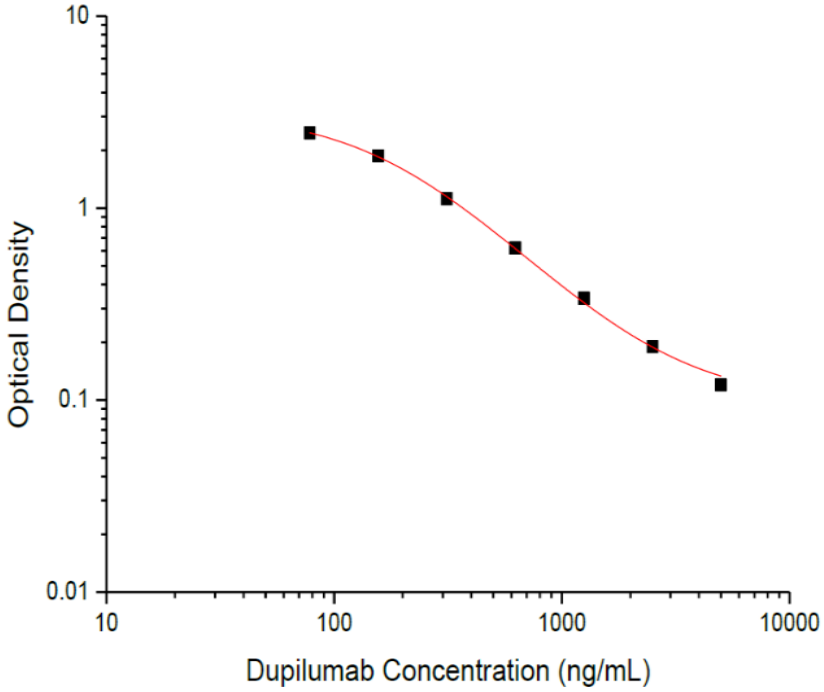
Average the duplicate readings for each standard and sample. Construct a standard curve by plotting the mean absorbance for each standard on the Y-axis against the concentration on the X-axis and draw a best fit curve through the points on the graph. Do not include the blank in the standard curve. The data may be linearized by plotting the log of the Dupilumab 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.

Typical Data & Standard Curve

The following assay data are provided for reference, since experimental environment and operation are different. The establishment of standard curve depends on your own assay.

STD. (ng/ml)	OD-1	OD-2	Average
0	3.48	3.50	3.49
78.13	2.45	2.47	2.46
156.25	1.84	1.90	1.87
312.5	1.10	1.14	1.12
625	0.61	0.62	0.62
1250	0.33	0.35	0.34
2500	0.19	0.18	0.19
5000	0.12	0.11	0.12



Performance

Sensitivity

The minimum detectable dose (MDD) of Dupilumab is typically less than 48.20 ng/mL.

The MDD was determined by subtracting two standard deviations to the mean O.D. value of twenty zero standard replicates and calculating the corresponding concentration.

Precision

Intra-Assay Precision (Precision within an assay): <20%

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

Inter-Assay Precision (Precision between assays): <20%

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

	Intra-Assay Precision			Inter-Assay Precision		
Sample	1	2	3	1	2	3
n	16	16	16	24	24	24
Mean (ng/mL)	2227.3	541.1	144.0	2228.6	542.0	128.1
Standard deviation	150.3	43.1	18.3	205.0	27.5	11.3
CV (%)	6.8	8.0	12.7	9.2	5.1	8.9

Recovery

The recovery of Dupilumab spiked to three different levels in human serum samples throughout the range of the assay was evaluated.

Sample	Dilution factor	Average (%)	Range (%)
Human serum (n=3)	1:10	99	92-103
	1:20	101	90-113

Linearity

To assess the linearity of the assay, human serum samples were spiked with high concentrations of Dupilumab and diluted with the Standard Diluent to produce samples with values within the dynamic range of the assay. (Human serum samples were prediluted 10-fold.

Sample	Dilution factor	Average (%)	Range (%)
Human serum (n=4)	1:2	90	83-94
	1:4	102	93-111

	1:8	111	108-115
	1:16	107	91-114

Stability

When the kit was stored at the recommended temperature for 6 months, the signal intensity decreased by less than 20%.

For unopened kits, if you want to prolong the storage time, please store the Standard, Detection A, Detection B and Microplate at - 20 ℃, the rest reagents should be store at 4℃.

ELISA Troubleshooting

High background/non-specific staining

Description of results	Possible reason	Recommendations and precautions
After termination, the whole plate results show a uniform yellow or light color; or the Standard curve is linear but the background is too high	The yellowing of the whole plate may be caused by wrong addition of other reagents	Check the components and lot numbers of the reagents before the experiment, and confirm that all components belong to the corresponding kit. Reagents from different kits or different lot numbers cannot be mixed.
	ELISA plate was not washed sufficiently	Make sure that the same amount of Washing Solution is added to each microwell during the washing process. After washing, press the ELISA plate firmly on the absorbent paper to remove the residual buffer.
	Incubation time too long	Please strictly follow the steps of the manual
	Streptavidin-HRP contaminates the tip and TMB container or positive control contaminates the Pre-coated Microplate	When absorbing different reagents, the tips should be replaced. When configuring different reagent components, different storage vessels should be used. Please use a pipette during operation.
	Biotinylated Antibody or Streptavidin-HRP concentration too high	Check whether the concentration calculation is correct or use after further dilution.
	Substrate exposure or contamination prior to use	Store in the dark at all times before adding substrate.
	Color development time is too long	Please strictly follow the steps of the manual.
	The wrong filter was used when the absorbance value was read	When TMB is used as the substrate, the absorbance should be read at 450 nm.

NO color plates

Description of results	Possible reason	Recommendations and precautions
After the color development step, all wells of the ELISA plate are colorless; the positive control is not obvious	Mixed use of component reagents	Please read labels clearly when preparing or using
	In the process of plate washing and sample enzyme contaminated addition, the marker is and inactivated, and loses its ability to catalyze the color developing agent	Confirm that the container holding the ELISA plate does not contain enzyme inhibitors (such as NaN_3, etc.) and confirm that the container for preparing the Wash Solution has been washed.
	Missing a reagent or a step	Review the manual in detail and strictly follow the operating steps

Light color

Description of results	Possible reason	Recommendations and precautions
The Standard is normal, the color of the sample is light	The sample uses NaN_3 preservative, which inhibits the reaction of the enzyme	Samples cannot use NaN_3
	The sample to be tested may not contain strong positive samples, so the result may be normal	In case of doubt, please test again.
The visual result is normal, but the reading value of the microplate reader is low	Wrong filter used for absorbance reading	When TMB is used as the substrate, the absorbance should be read at 450 nm.
All wells, including Standard and Samples, are lighter in color	Insufficient incubation time	Timer accurate timing
	Insufficient color reaction	Usually 15 - 30 minutes
	The number of washings increases, and the dilution ratio of the concentrated lotion does not meet the requirements	Reduce the impact of washing, dilute the concentrated lotion and washing time according to the manual, and accurately record the washing times and dosage.
	Distilled water quality problem	The prepared lotion must be tested to see if the pH value is neutral.
	In the process of plate washing and sample addition, the enzyme marker is contaminated and inactivated, and loses its ability to catalyze the color developing agent.	Confirm that the container holding the ELISA plate does not contain enzyme inhibitors (such as NaN_3, etc.), confirm that the container for preparing the Washing Solution has been washed, and confirm that the purified water for preparing the Washing Solution meets the requirements and is not contaminated.

	The kit has expired or been improperly stored	Please use it within the expiration and store it in accordance with the storage conditions recommended in the manual to avoid contamination.
	Reagents and samples are not equilibrated before use	All reagents and samples should be equilibrated at room temperature for about 30 minutes.
	Insufficient suction volume of the pipette, too fast discharge of pipetting suction, too much liquid hanging on the inner wall of the tip or the inner wall is not clean.	To calibrate the pipette, the tips should be matched, each time the tips should fit tightly, the pipetting should not be too fast, and the discharge should be complete. The inner wall of the tips should be clean, and it is best to use it once.
Poor repeatability	Incubation temperature constant temperature effect is not good	Keep the temperature constant to avoid the local temperature being too high or too low
	When adding liquid, too much remains on the medial wall of wells	When adding liquid, the tip should try to add liquid along the bottom of the medial wall of wells without touching the bottom of the hole.
	Reuse of consumables	The tips should be replaced when different reagents are drawn, and different storage vessels should be used when configuring different reagent components.
	The bottom of the microwell is scratched or there is dirt	Be careful when operating, be careful not to touch the bottom and wipe the bottom of the microplate to remove dirt or fingerprints.
		Technical repetition of the same sample for 3 times, including more than 2 approximate values.
	Cross-contamination during sample addition	Try to avoid cross-contamination when adding samples
The color of plate is chaotic and irregular	Cross-contamination from manual plate washing	When washing the plates by hand, the first 3 injections of the lotion should be discarded immediately, and the soaking time should be set for the next few times to reduce cross-contamination.
	Cross-contamination when clapping	Use a suitable absorbent paper towel when clapping the plate, do not pat irrelevant substances into the well of the plate, and try not to pat in the same position to avoid cross-contamination.

Description of results	Possible reason	Recommendations and precautions

The color of plate is chaotic and irregular	The liquid filling head of the plate washer is blocked, resulting in unsatisfactory liquid addition or large residual amount of liquid suction, resulting in the color of plate is chaotic and irregular	Unblock the liquid addition head, so that each well is filled with washing liquid when washing the plate and the residual amount should be small when aspirating liquid.
	Incomplete centrifugation of the sample, resulting in coagulation in the reaction well or interference of sediment or residual cellular components	Serum plasma should be fully centrifuged at 3000 rpm for more than 6 minutes
	The sample is stored for too long time, resulting in contamination.	Samples should be kept fresh or stored at low temperature to prevent contamination
	Incorrect preparation of Washing Solution or direct misuse of concentrated Washing Solution	Please configure according to the manual

Declaration

1. Limited to current conditions and scientific techniques, all raw materials are not completely identified and analyzed. This product may have a technology-related quality risk.
2. During the ELISA kit development, some endogenous interferons (not all) in the biological sample have been removed or decreased.
3. The final assay result is related to the validity of reagents, experimental operation and environment. We are only responsible for this kit, excluding sample consumption during using this kit. Before use, please consider and prepare enough samples required by the assay.
4. To get a satisfied assay result, please use all reagents offered by this kit. Don't use any product from other vendors. Strictly follow instructions of this manual.
5. During assay procedure, incorrect reagents preparation and parameter setting of the microplate reader may result in the abnormal result. Before assay, please read this manual carefully and adjust instruments.
6. Even if the assay is performed by the same person, results in two independent assays may be different. Thus, each step in the assay should be controlled to ensure the reproducibility.
7. Before delivery, this kit is subject to the strict QC. Influenced by transportation conditions and experimental devices, the assay result got by the customer may be different from original data. Inter-assay CV between different batches may be caused by reasons before.
8. This kit is not compared to similar kits from other vendors or methods for testing the same detection target. Thus, assay results may be inconsistent.
9. This kit allows for research use only. For IVD or other purposes, we are not responsible for relevant consequences and doesn't bear any legal liability.

Precautions for Kits

1. When using different ELISA kits, labelling is required to avoid mixed components and failed assay.
2. After opening the kit, please refer to the table of storage condition for coated plate and standards (Dampness may decrease the activity.). If any component is missing or damaged during the assay or storage, please contact us for ordering a new one to replace. (e.g. lyophilized standard)
3. Sterile and disposable tips are required during the assay. After use, the reagents bottle cap has to be tightened to avoid the microbial contamination and evaporation.
4. While manual washing, please keep tips or pipettors for adding wash buffer away from the well. Insufficient washing or contamination easily causes false positive and high background.
5. During the assay, prepare required reagents for next step in advance. After washing, add the reagent into the well in time to avoid dryness. Otherwise, dry plate will result in the failed assay.
6. Before confirmation, reagents from other batches or sources should not be used in this kit.
7. Don't reuse tips and tubes to avoid cross contamination.
8. After loading, seal the plate to avoid the evaporation of the sample during incubation. Complete the incubation process at recommended temperature.
9. Please wear the lab coat, mask and gloves to protect yourself during the assay. Especially, for the detection of blood or other body fluid samples, please follow regulations on safety protection of biological laboratory.

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