Human Ubiquitin Conjugating Enzyme E2C (UBE2C) ELISA Kit

Cat #: orb776993 (manual)

For research use only. Not intended for diagnostic use.

Product Features

Detectable Sample Type: Tissue homogenates, cell lysates and other biological fluids.

Sensitivity: 0.062 ng/mL

Detection Range: 0.16-10 ng/mL

Specificity: This assay has high sensitivity and excellent specificity for detection of Human UBE2C. No significant cross-reactivity or interference between Human UBE2C and analogues was observed.

Internal Test Data: N/A

Assay Principle

This kit uses a sandwich enzyme immunoassay principle. The microtiter plate is pre-coated with an antibody specific to Human UBE2C. Standards or samples are added to the wells, followed by a biotin-conjugated antibody specific for Human UBE2C. Avidin conjugated to horseradish peroxidase (HRP) is then added and incubated. After addition of the TMB substrate, color develops only in wells containing Human UBE2C bound to the biotin-conjugated antibody and HRP-avidin complex. The reaction is stopped with sulfuric acid, and absorbance is measured at 450 nm ± 10 nm. Human UBE2C concentrations are determined by comparison with a standard curve.

Kit Components

Reagents	Quantity		Storage Condition
	48T	96T	
Pre-Coated Microplate	6 strips x 8 wells	12 strips x 8 wells	4°C/-20°C
Standard (Lyophilized)	1 vial	2 vials	4°C/-20°C
Biotinylated Antibody (100×)	60 µL	120 µL	4°C/-20°C
Streptavidin-HRP (100×)	60 µL	120 µL	4°C/-20°C
Standard/Sample Diluent Buffer	10 mL	20 mL	4°C/-20°C
Biotinylated Antibody Diluent	6 mL	12 mL	4°C/-20°C
HRP Diluent	6 mL	12 mL	4°C/-20°C
Wash Buffer (25×)	10 mL	20 mL	4°C/-20°C
TMB Substrate Solution	6 mL	10 mL	4°C/-20°C (store in dark)

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Stop Reagent	3 mL	6 mL	4°C/-20°C
Plate Covers	1 piece	2 pieces	RT

Special Explanation

1. *If the kit has been opened, store the entire kit at 4 °C. If the kit is not fully used within one week, store the Pre-Coated Microplate, Standard, Biotinylated Antibody, and Streptavidin-HRP at −20 °C, and store the remaining reagents at 4 °C. All components should be used within 6 months.
2. *If the kit is unopened, store the entire kit at 4 °C for short-term storage (valid for 6 months) or at −20 °C for long-term storage (valid for 1 year). Avoid repeated freeze–thaw cycles.
3. Do not use the kit beyond the expiration date.
4. If the entire kit is stored at −20 °C, transfer it to 4 °C one day before the experiment.
5. After opening the package, verify that all components are present and intact.
6. Ensure all caps are tightly closed to prevent evaporation and microbial contamination. Reagent volumes may be slightly greater than the amounts indicated on the labels; use accurate measuring equipment and do not pour directly from the vial.

All kit components have been formulated and quality-control tested to function properly as a complete system. Do not mix or substitute reagents or materials from other kits, as this may compromise assay performance.

Materials Required but Not Supplied

1. Microplate reader capable of measuring absorbance at 450 ± 10 nm.
2. High-speed centrifuge.
3. Electro-heating standing-temperature cultivator.
4. Absorbent paper.
5. Double distilled water or deionized water.
6. Single or multi-channel pipettes with high precision and disposable tips.
7. Precision pipettes to deliver 2 µL to 1 mL volumes.

Sample Preparation

1. Equilibrate all materials and prepared reagents to room temperature prior to use. Prior to use, mix all reagents thoroughly taking care not to create any foam within the vials.
2. The user should calculate the possible amount of the samples used in the whole test. Please reserve sufficient samples in advance.

Please predict the concentration before assaying. If values for these are not within the range of the Standard curve, users must determine the optimal sample dilutions for their particular experiments.

Sample Collection and Storage

Serum – Collect samples in a serum separator tube. Allow blood to clot for 2 hours at room temperature or overnight at 4 °C, then centrifuge at 1000 × g for 20 minutes. Assay freshly prepared serum immediately or aliquot and store at −20 °C or −80 °C for later use. Avoid repeated freeze–thaw cycles.

Plasma – Collect plasma using EDTA or heparin as the anticoagulant. Centrifuge within 30 minutes of collection at 1000 × g and 2–8 °C for 15 minutes. Remove plasma and assay immediately or aliquot and store at −20 °C or −80 °C for later use. Avoid repeated freeze–thaw cycles.

Tissue homogenates - Preparation varies depending on tissue type.

1. Rinse tissues thoroughly with pre-cooled PBS to remove excess blood, then weigh prior to homogenization.

2. Mince tissues into small pieces and homogenize in fresh lysis buffer (the choice of buffer depends on the subcellular location of the target protein; PBS may be used for most tissues) at a weight-to-volume ratio of 1:9 (e.g., 900 µL lysis buffer per 100 mg tissue) using a glass homogenizer on ice (micro tissue grinders may also be used).

3. Sonicate the suspension with an ultrasonic cell disruptor until the solution becomes clear.

4. Centrifuge the homogenate at 10,000 × g for 5 minutes, collect the supernatant, and assay immediately or aliquot and store at ⩽ −20 °C.

Note: It is recommended to measure protein concentration in tissue homogenates simultaneously to obtain a more accurate concentration of the target substance per milligram of protein.

Cell lysates - Cells must be lysed prior to assay.

1. Wash adherent cells gently with pre-cooled PBS, detach with trypsin, and collect by centrifugation at 1000 × g for 5 minutes (suspension cells may be collected directly by centrifugation).

2. Wash cells three times with pre-cooled PBS.

3. Resuspend cells in fresh lysis buffer at a concentration of 10^7 cells/mL. If necessary, sonicate until the solution becomes clear.

4. Centrifuge at 1500 × g for 10 minutes at 2–8 °C to remove cellular debris. Assay immediately or aliquot and store at ⩽ −20 °C.

Urine - Collect the first midstream urine of the day into a sterile container. Centrifuge to remove particulate matter, then assay immediately or aliquot and store at ⩽ −20 °C. Avoid repeated freeze–thaw cycles.

Saliva - Collect saliva using a collection device or equivalent method. Centrifuge at 1000 × g and 2–8 °C for 15 minutes to remove particulates. Assay immediately or aliquot and store at ⩽ −20 °C. Avoid repeated freeze–thaw cycles.

Feces - Collect dried fecal samples whenever possible, weighing more than 50 mg. Wash feces three times with PBS at a weight-to-volume ratio of 1:9 (e.g., 900 µL lysis buffer per 100 mg feces), then sonicate (or mash) and centrifuge at 5000 × g for 10 minutes. Collect the supernatant for analysis.

Cell culture supernatants and other biological fluids - Centrifuge samples at 1000 × g for 20 minutes. Collect the supernatant and assay immediately or store samples in aliquot at -20°C or -80°C for later use. Avoid repeated freeze-thaw cycles.

Cerebrospinal fluid (CSF) - Centrifuge samples at 1000 × g for 20 minutes. Collect the supernatant and assay immediately, or aliquot and store at −20 °C or −80 °C for later use. Avoid repeated freeze–thaw cycles.

Sample Dilution Proposal

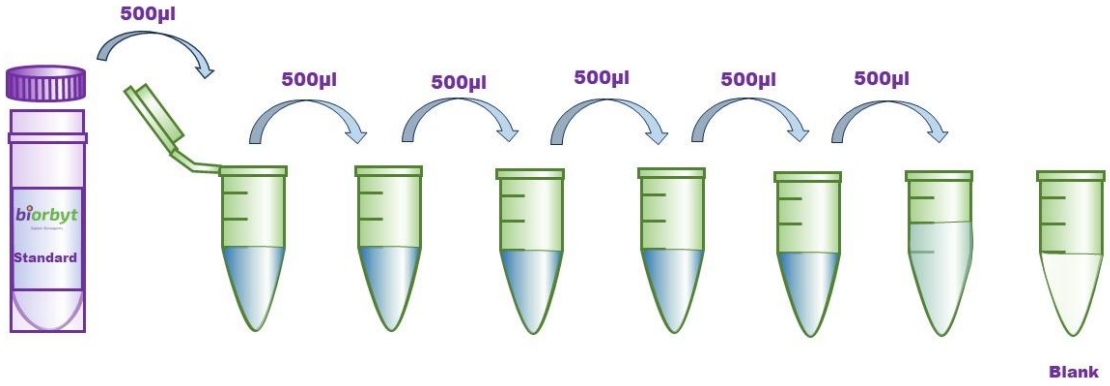
1. Fresh, normal serum or plasma samples are recommended for testing without dilution.
2. Due to individual variation, the recommended dilution ratios are for reference only. It is advised to perform a preliminary experiment to determine the optimal dilution ratio.

Notes

1. Samples intended for use within 5 days may be stored at 4 °C. Otherwise, samples should be stored at −20 °C (≤ 1 month) or −80 °C (≤ 2 months) to prevent loss of bioactivity and contamination. Avoid repeated freeze–thaw cycles.
2. Hemolyzed samples may affect assay results and should not be used.
3. Bring samples to room temperature before performing the assay.

Reagent Preparation

1. Bring all kit components and samples to room temperature (18–25 °C) before use. Ensure that all components are fully dissolved and thoroughly mixed prior to use.
2. If the kit will not be used in a single experiment, remove only the strips and reagents required for the current assay and store the remaining components as specified.
3. Dilute the 25× Wash Buffer to 1× with double-distilled water.
4. **Standard Working Solution** - Centrifuge the Standard at 1000 × g for 1 minute. Reconstitute the Standard with 1.0 mL of Standard Diluent Buffer, allow it to stand at room temperature for 10 minutes, and mix gently without foaming. The concentration of the reconstituted stock Standard is 10 ng/mL. Prepare 7 tubes, each containing 0.5 mL of Standard Diluent Buffer, and use the Standard to generate a two-fold serial dilution as illustrated below. Mix each tube thoroughly by pipetting up and down several times before transferring to the next tube. Prepare standards at 10 ng/mL, 5 ng/mL, 2.5 ng/mL, 1.25 ng/mL, 0.63 ng/mL, 0.32 ng/mL, 0.16 ng/mL. The final tube containing only Standard Diluent serves as the **blank** (0 pg/mL). To ensure assay validity, prepare a fresh Standard solution for each experiment. When diluting from higher to lower concentrations, use a new pipette tip for each step. Note: The last tube is used as the **blank** and should not receive solution from the preceding tube.



10 ng/mL	5	2.5	1.25	0.63	0.32	0.16

5. **1× Biotinylated Antibody and 1× Streptavidin-HRP -** Briefly spin or centrifuge the stock Biotinylated Antibody and Streptavidin-HRP before use. Dilute each reagent 100-fold to the working concentration using the corresponding Biotinylated Antibody Diluent and HRP Diluent.
6. **TMB Substrate Solution** - Withdraw the required volume using sterile pipette tips. Do not return any unused solution to the original vial.

Notes

1. After receiving the kit, store all reagents according to the instructions. The microplate can be separated into individual strips and used in batches as needed.

2. All test tubes, pipette tips, and reagents used in the experiment are disposable and must not be reused, as this may affect the results. Reagents from different kit lots must not be mixed, except for TMB Substrate, Wash Buffer, and Stop Reagent.

3. Lyophilized Standards, Biotinylated Antibody, and Streptavidin-HRP are low in volume and may adhere to the tube walls during transport. Centrifuge at 1000 × g for 1 minute before use, then gently pipette 4–5 times to mix. Prepare the working solutions according to the required volume using the corresponding dilution buffers; do not mix different dilution solutions.

4. Bring all reagents to room temperature (18–25 °C) before use. Crystal formation in the 25× concentrate is normal. Warm to room temperature (not exceeding 40 °C) and mix gently until crystals are completely dissolved.

5. Reconstitute the Standard within 15 minutes before the assay. The Standard Working Solution is for single use only and should be discarded if not fully used. Sample addition should be performed quickly, preferably within 10 minutes. To ensure accuracy, it is recommended to run samples in duplicate. When pipetting reagents, maintain a consistent order of addition to ensure equal incubation times for all wells.

6. During washing, remove residual wash buffer by gently tapping the plate on absorbent paper. Do not place the paper directly into the wells. Before reading, ensure that residual liquid and fingerprints are removed from the bottom of the plate to avoid interference with the microplate reader.

7. TMB Substrate Solution is light-sensitive; avoid prolonged exposure to light. Dispense TMB within 15 minutes after washing the microtiter plate. Avoid contact between TMB and metal to prevent premature color development. If TMB turns blue before use, it is contaminated and should be discarded. TMB is toxic; avoid direct contact with skin.

8. Bacterial or fungal contamination of samples or reagents, or cross-contamination between reagents, may result in inaccurate results.

Assay Procedure

1. Determine the wells for the Diluted Standards, Blank, and Samples. Prepare 7 wells for the Standards and 1 well for the Blank. Add 100 µL of each Standard Working Solution (see *Reagent Preparation*) or 100 µL of sample to the appropriate wells. Cover with the plate cover and incubate for 80 minutes at 37 °C. Note: Add solutions to the bottom of each ELISA plate well. Avoid touching the inner wall of the wells and minimize foaming.

2. Discard the liquid from each well. Aspirate and wash each well with 200 µL of 1× Wash Solution, allowing it to sit for 1–2 minutes. Remove all remaining liquid by firmly tapping the plate onto absorbent paper. Wash a total of 3 times. After the final wash, remove any residual Wash Buffer by aspiration or decanting. Invert the plate and blot against absorbent paper.

Notes:
(a) When adding Wash Solution, do not allow the pipette tip to touch the well walls to avoid contamination.
(b) When dispensing wash buffer, ensure it is delivered directly into the wells to prevent cross-contamination.

3. Add 100 µL of Biotinylated Antibody Working Solution to each well. Cover with the plate cover and incubate for 50 minutes at 37 °C.

4. Repeat the aspiration and wash procedure a total of 3 times as described in Step 2.

5. Add 100 µL of Streptavidin-HRP Working Solution to each well. Cover with the plate sealer and incubate for 50 minutes at 37 °C.

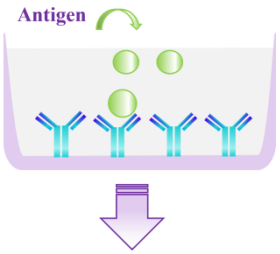
6. Repeat the aspiration and wash procedure a total of 5 times as described in Step 2.

7. Add 90 µL of TMB Substrate Solution to each well. Cover with a new plate cover and incubate for 20 minutes at 37 °C (do not exceed 30 minutes) in the dark. The solution will turn blue after TMB addition. Preheat the microplate reader for approximately 15 minutes before measuring absorbance.

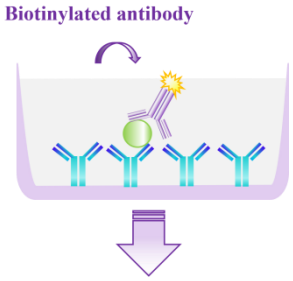
8. Add 50 µL of Stop Reagent to each well. The solution will turn yellow upon addition. Mix by gently tapping the side of the plate. If the color change is not uniform, tap gently again to ensure thorough mixing. Add the Stop Reagent in the same order as the TMB Substrate Solution.

9. Wipe away any water droplets or fingerprints from the bottom of the plate and ensure there are no bubbles on the liquid surface. Immediately read the plate at 450 nm using a microplate reader.

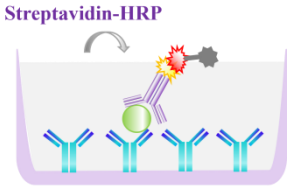
Assay Procedure Summary



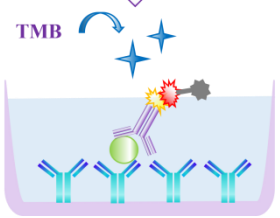
1. After the kit has equilibrated to room temperature, add 100 µL of Standard Working Buffer (serially diluted according to the instructions) or 100 µL of sample to each well. Incubate at 37 °C for 80 minutes.



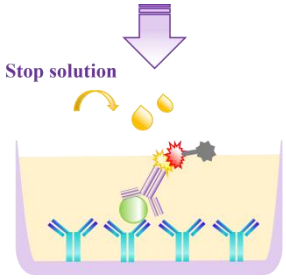
2. Discard the liquid from the plate, add 200 µL of 1× Wash Buffer to each well, and wash the plate 3 times. After blotting the plate dry on clean absorbent paper, add 100 µL of 1× Biotinylated Antibody Working Solution to each well and incubate at 37 °C for 50 minutes.



3. Discard the liquid from the plate, add 200 µL of 1× Wash Buffer to each well, and wash the plate 3 times. After blotting the plate dry on clean absorbent paper, add 100 µL of 1× Streptavidin-HRP Working Solution to each well and incubate at 37 °C for 50 minutes.



4. Discard the liquid from the plate, add 200 µL of 1× Wash Buffer to each well, and wash the plate 5 times. After blotting the plate dry on clean absorbent paper, add 90 µL of TMB Substrate Solution to each well and incubate at 37 °C for 20 minutes in the dark.

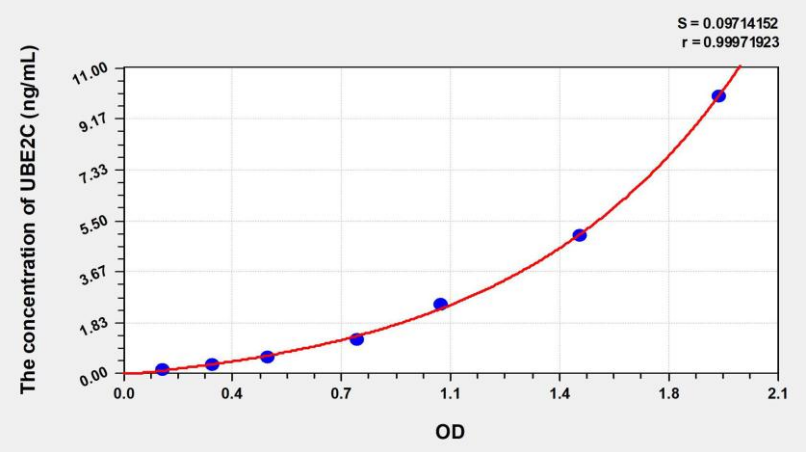


5. Add 50 µL of Stop Solution to each well and shake the plate on a plate shaker for 1 minute to mix. Record the OD at 450 nm immediately, calculation of the results.

Calculation of Results

Average the duplicate readings for each Standard, Control, and Sample, and subtract the mean optical density of the zero Standard. Construct a standard curve by plotting the Human UBE2C concentration on the y-axis against absorbance on the x-axis and draw a best-fit curve through the data points. If samples were diluted, multiply the concentration obtained from the standard curve by the dilution factor. Curve-fitting software, such as Curve Expert, may be used for data analysis.

Concentration (ng/mL)	OD	Corrected OD
10	2.027	1.946
5	1.576	1.495
2.5	1.118	1.037
1.25	0.847	0.766
0.63	0.553	0.472
0.32	0.372	0.291
0.16	0.211	0.13
0	0.081	0.000



Note: this graph is for reference only

Performance

Precision

Intra-assay Precision (Precision within an assay): **CV% < 8%**

Three samples of known concentration were tested twenty times on 1 plate to assess intra-assay precision.

Inter-assay Precision (precision between assays): CV**% < 10%**

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

Recovery

The matrices listed below were spiked with certain level of recombinant Human UBE2C and the recovery rates were calculated by comparing the measured value to the expected amount of Human UBE2C in samples.

Matrix	Recovery range	Average
Serum (n = 5)	80-95%	87%
EDTA plasma (n = 5)	78-96%	87%
Heparin plasma (n = 5)	78-92%	85%

Linearity

The linearity of the kit was evaluated by testing samples spiked with appropriate concentrations of Human UBE2C and their serial dilutions. Results were expressed as the percentage of the measured concentration relative to the expected concentration.

Sample	1:2	1:4	1:8	1:16
Serum (n = 5)	86-94%	81-95%	98-105%	87-98%
EDTA plasma (n = 5)	95-102%	78-97%	86-97%	81-103%
Heparin plasma (n = 5)	88-97%	85-92%	83-96%	93-102%

ELISA Troubleshooting

High background/non-specific staining

Description of results	Possible reason	Recommendations and precautions
After stopping the reaction, the entire plate shows a uniform yellow or light color, or the standard curve is linear but the background signal is excessively high.	Yellowing of the entire plate may be caused by incorrect addition of reagents.	Before starting the experiment, verify the components and lot numbers of all reagents to ensure they belong to the same kit. Reagents from different kits or different lot numbers must not be mixed.
	The ELISA plate was not washed sufficiently.	Ensure that the same volume of Wash Solution is added to each microwell during the washing process. After washing, firmly tap the ELISA plate on absorbent paper to remove any residual buffer.
	Incubation time was too long.	Strictly follow the procedures outlined in the manual.

	Streptavidin-HRP contaminated the pipette tip or TMB container, or the positive control contaminated the pre-coated microplate.	Replace pipette tips when dispensing different reagents. Use separate containers when preparing different reagent components, and always use a pipette during handling.
	The concentration of Biotinylated Antibody or Streptavidin-HRP was too high.	Verify that concentration calculations are correct, or perform further dilution if necessary.
	The substrate was exposed to light or contaminated prior to use.	Store reagents in the dark at all times prior to substrate addition.
	Color development time was too long.	Strictly follow the procedures outlined in the manual.
	An incorrect filter was used when reading the absorbance value.	When TMB is used as the substrate, measure absorbance 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, and the positive control is not clearly detectable. s	Components from different reagent sets were mixed.	Carefully read reagent labels when preparing or using them.
	During plate washing or sample/enzyme addition, the enzyme label was contaminated or inactivated, resulting in loss of its ability to catalyze color development.	Confirm that the container holding the ELISA plate does not contain enzyme inhibitors (such as NaN_3), and ensure that the container used to prepare the Wash Solution has been thoroughly cleaned.
	A reagent or procedural step was omitted.	Review the manual carefully and strictly follow the operating procedures.

Light color

Description of results	Possible reason	Recommendations and precautions
The Standard appears normal, but the sample color is weak.	The sample contains NaN_3 preservative, which inhibits the enzyme reaction.	Samples must not contain NaN_3.
	The sample being tested may not contain strongly positive material, so the result may be normal.	If there is any doubt about the results, repeat the assay.
The visual result appears normal, but the microplate reader values are low.	An incorrect filter was used for absorbance measurement.	When TMB is used as the substrate, absorbance should be measured at 450 nm.
	Insufficient incubation time.	Ensure accurate timing using a timer.

All wells, including Standards and Samples, show weak color development.	Inadequate color development.	Typically 15–30 minutes.
	Excessive washing, or the dilution ratio of the concentrated Wash Buffer does not meet requirements.	Minimize the impact of washing by diluting the concentrated Wash Buffer and setting the washing time according to the manual. Accurately record the number of washes and the volume used.
	Poor quality distilled water.	The prepared Wash Buffer should be tested to confirm that the pH is neutral.
	During plate washing or sample addition, the enzyme label was contaminated or inactivated, resulting in loss of catalytic activity.	Confirm that the container holding the ELISA plate does not contain enzyme inhibitors (such as NaN_3), that the container used to prepare the Wash Buffer has been thoroughly cleaned, and that the purified water used meets the required standards and is not contaminated.
	The kit has expired or was improperly stored.	Use the kit within its expiration date and store it according to the conditions recommended in the manual to avoid contamination.
	Reagents and samples were not equilibrated to room temperature before use.	Allow all reagents and samples to equilibrate at room temperature for approximately 30 minutes before use.
	Insufficient pipetting volume, overly rapid dispensing, excessive liquid remaining on the inner wall of the tip, or unclean tip walls.	Calibrate the pipette properly. Ensure tips are compatible and fit securely, pipette at an appropriate speed, and fully dispense the liquid. Tips should have clean inner walls and be used only once
Poor repeatability.	Incubation temperature was not properly controlled.	Maintain a constant incubation temperature and avoid localized temperature extremes.
	Excess liquid remained on the inner wall of the wells during liquid addition.	When adding liquids, dispense along the lower inner wall of the wells without touching the bottom.
	Reuse of consumables.	Replace pipette tips when drawing different reagents, and use separate containers when preparing different reagent components.
	The bottom of the microwell is scratched or contaminated.	Handle the plate carefully. Avoid touching the well bottoms and clean the bottom of the microplate to remove dirt or fingerprints.
		Perform technical replicates of the same sample three times, ensuring that at least two values are comparable.
	Cross-contamination during sample addition.	Minimize the risk of cross-contamination during sample addition.
	Cross-contamination during manual plate washing.	When washing plates manually, discard the first three washes immediately, then

The color development across the plate is uneven and irregular.		allow soaking during subsequent washes to reduce cross-contamination.
	Cross-contamination during plate tapping.	Use appropriate absorbent paper when tapping the plate. Avoid introducing foreign material into the wells, and avoid tapping in the same position repeatedly to reduce cross-contamination.

Description of results	Possible reason	Recommendations and precautions
The color development of the plate is uneven and irregular	The liquid dispensing head of the plate washer is clogged, leading to improper liquid dispensing or excessive residual liquid after aspiration, which results in uneven and irregular color development across the plate.	Unclog the liquid dispensing head to ensure that each well is properly filled with wash solution during plate washing and that minimal residual liquid remains after aspiration.
	Incomplete centrifugation of the sample, resulting in coagulation within the reaction wells or interference from sediment or residual cellular components.	Serum and plasma samples should be fully centrifuged at 3000 rpm for more than 6 minutes.
	The sample was stored for an excessively long period, leading to contamination.	Samples should be kept fresh or stored at low temperatures to prevent contamination.
	Incorrect preparation of the Wash Solution or direct use of the concentrated Wash Solution.	Prepare all reagents strictly according to the manual.

Declaration

1. For research use only. Not intended for diagnostic use.
2. The kit may not be suitable for special experimental samples in which the validity of the experiment is uncertain, such as gene knockout experiments.
3. Certain natural or recombinant proteins, including both prokaryotic and eukaryotic recombinant proteins, may not be detected if they do not match the capture and detection antibodies used in this product.

This kit has not been compared with similar kits from other manufacturers or with products that use different methods to detect the same target. Therefore, differences in test results cannot be ruled out.

Safety Notes

1. This kit is intended for laboratory research and development use only and must not be used in humans or animals.
2. Reagents should be considered hazardous substances and must be handled with appropriate care.
3. Gloves, lab coats, and safety goggles should always be worn to prevent skin and eye contact with the Stop Solution and TMB. In case of contact, rinse thoroughly with water.

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