Mouse Beta Actin (ACTB) ELISA Kit

Cat #: orb3011990 (manual)

Size: 96 tests / 48 tests

For research use only. Not intended for diagnostic use.

Product Features

Detection Range: 1.56-100ng/mL

Sensitivity: 0.76ng/mL

Sample Types: serum, plasma, tissue homogenates, cell lysates, cell culture supernates and other biological fluids

Specificity: Detects mouse ACTB in samples with no significant cross-reactivity with its analogues.

Background:

ACTB Introduction

β-actin (ACTB) is one of six mouse actin isoforms and one of two non-muscle cytoskeletal actins. Actins are highly conserved proteins essential for cell motility, structure, and integrity. In contrast, α-actin is a key component of the muscle contractile apparatus.

Assay Principle

This kit is based on double antibody sandwich enzyme-linked immunosorbent assay (ELISA). Samples, standards, biotinylated detection antibodies, and HRP enzyme conjugates were added to the microwells pre-coated with mouse ACTB capture antibody, incubated and washed, and then developed using substrate TMB. TMB is converted to blue under the catalysis of peroxidase (HRP) and then transformed into the final yellow under acidic conditions. The shade of colour was positively correlated with the mouse ACTB in the sample. Measure the absorbance (OD value) with a microplate reader at a wavelength of 450 nm and calculate the sample concentration.

Kit Components

Reagents	Specifications（96T）	Specifications（48T）	Storage Conditions
Pre-coated Assay Plate	8×12	8×6	2-8°C
Standard	2 tubes	1 tube	2-8°C
Universal Diluent	2×20 mL	1×20 mL	2-8°C
Biotin-antibody (100×)	120 µl	60 µl	2-8°C
Streptavidin-HRP (100×)	120 µl	60 µl	2-8°C
Wash Buffer (20×)	2×10 mL	1×10 mL	2-8°C

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TMB Substrate	10 ml	5 ml	2-8°C
Stop Solution	6 ml	3 ml	2-8°C
Plate Sealer	4 pieces	4 pieces	
Manual	1 copy	1 copy	

Materials Required but Not Supplied

1. Microplate reader (wavelength: 450nm)
2. Precision pipette with disposable tips: 0.5-10uL, 5-50uL, 20-200uL, 200-1000uL
3. 37°C incubator
4. Deionized or distilled water

Sample Preparation

Sample Collection and Requirements

1. **The detection range of the kit is not equivalent to the concentration range of the analyte in the sample,** and it is recommended to estimate the concentration of the analyte in the sample through relevant literature before the experiment and determine the actual concentration of the sample through the pre-experiment Condition. If the concentration of analyte in the sample is too high or too low, dilute or concentrate the sample appropriately.

2. If the sample to be tested is not among the samples listed in the manual, it is recommended to do a pre-test to verify the validity of the test.

3. **Serum:** Whole blood specimens collected in serum separators are stored at room temperature for 2 hours or 4°C overnight, then centrifuge at 1000×g for 20 minutes, and the supernatant can be taken or stored at -20°C or -80°C, but repeated freeze-thaw should be avoided.

4. **Plasma:** Collect specimens with EDTA or heparin as anticoagulants and centrifuge the specimen at 1000×g at 2-8°C for 15 minutes within 30 minutes of collection, take the supernatant for detection, or store the supernatant at -20°C or -80°C, but avoid repeated freeze-thaw cycles.

5. **Tissue homogenate:** Rinse the tissue with pre-chilled PBS (0.01M, pH=7.4) to remove residual blood (lysed red blood cells in the homogenate can affect the measurement) and mince the tissue after weighing. The minced tissue is compared to the corresponding volume of PBS (generally according to the weight-to-volume ratio of 1:9, for example, 1g of tissue sample corresponds to 9mL of PBS, the specific volume can be adjusted appropriately according to the needs of the experiment, and a record should be made. It is recommended to add protease inhibitors to PBS) to a glass homogenizer and grind well on ice. To further lyse the histiocytes, the homogenate can be sonicated or repeatedly freeze-thaw. Finally, the homogenate was centrifuged at 5000×g for 5~10 minutes, and the supernatant was taken for detection.

6. **Cell culture supernatant:** Centrifuge at 1000×g for 20 minutes to detect or store the supernatant at -20°C or -80°C but avoid repeated freeze-thaw cycles.

7. **Cell lysate**: For adherent cells, gently wash with pre-cooled PBS, then digest with trypsin, and collect the cells by centrifuging at 1000×g for 5 minutes; suspended cells can be collected directly by centrifugation. Wash the collected cells with pre-cooled PBS 3 times and resuspend 150-200μL PBS for every 1×106 cells (it is recommended to add a protease inhibitor in the PBS; if the content is very low, you can appropriately reduce the volume of PBS) and lyse the cells by repeated freeze-thaw cycles or

sonication. Centrifuge the extracted solution at 1500×g for 10 minutes at 2-8°C and collect the supernatant for detection.

8. **Other biological specimens:** Centrifuge at 1000×g for 20 minutes and take the supernatant for detection.

9. **Appearance of the sample:** The sample should be clear and transparent, and the suspended solids should be centrifuged and removed.

10. **Sample preservation:** If the sample is tested within 1 week after collection, it can be stored at 4°C, if it cannot be tested in time, please divide it into one-time use and freeze it at -20°C (test within 1 month), or -80°C (testing within 6 months), avoid repeated freezing and thawing, hemolysis of the specimen will affect the final test result, so hemolyzed specimens should not be tested for this test.

Recommended Sample Dilution Ratio

Please estimate the concentration range of the samples in advance. If your test sample needs to be diluted, refer to the dilution protocols below.

Dilution 100 times: One-step dilution. Take 5μL of the sample and add it to 495μL of universal diluent to make a 100-fold dilution

Dilution 1000 times: Two-step dilution. Take 5μL of the sample and add it to 95μL of universal diluent to make a 20-fold dilution, then take 5μL of the 20-fold diluted sample and add it to 245μL of universal diluent to make a 50-fold dilution, resulting in a total dilution of 1000 times

Dilution 100000 times: Three-step dilution. Take 5μL of the sample and add it to 195μL of universal diluent to make a 40-fold dilution, then take 5μL of the 40-fold diluted sample and add it to 245μL of universal diluent to make a 50-fold dilution, and finally take 5μL of the 2000-fold diluted sample and add it to 245μL of universal diluent to make a 50-fold dilution, resulting in a total dilution of 100000 times;

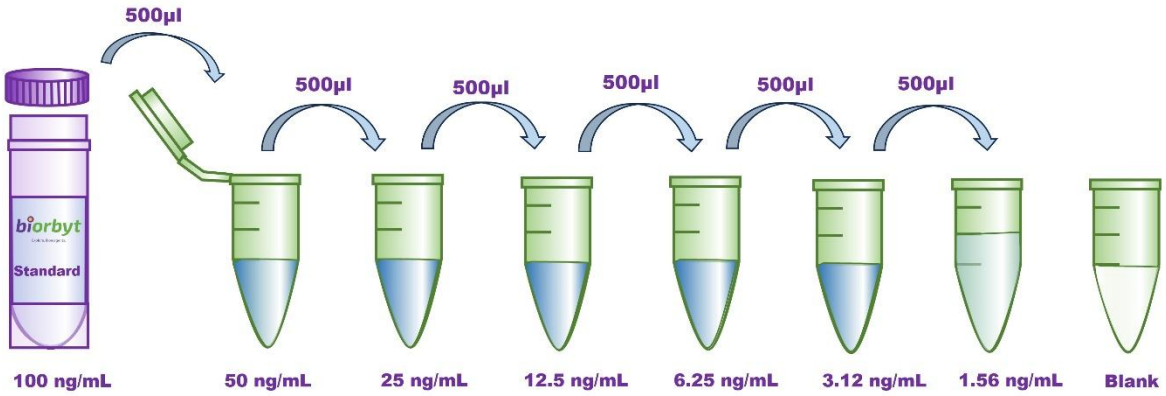
For each dilution step, the volume taken should be **no less than 3μL**, and the dilution factor should not exceed 100 times. Each dilution step must be mixed thoroughly to avoid foaming.

Reagent Preparation

1. Remove the kit from the refrigerator 10 minutes in advance and equilibrate to room temperature.

2. **Standard gradient working solution:** Add 1mL of universal dilution to the lyophilized standard, let it stand for 15 minutes, let it completely dissolve, and then mix gently (at a concentration of 100 ng/mL), then dilute at the following concentrations: 100ng/mL, 50ng/mL, 25ng/mL, 12.5ng/mL, 6.25ng/mL, 3.12ng/mL, 1.56ng/mL, 0ng/mL.

Doubling dilution method: take 7 EP tubes, add 500μL of general dilution solution to each tube, and draw 500μL from 100ng/mL standard working solution to the first EP tube to mix well to prepare 50 ng/mL standard working solution, and then pipette and mix well according to this step. The last tube is used directly as a blank well, and there is no need to draw liquid from the penultimate tube, as shown in the figure below.



3. **Preparation of biotinylated antibody detection solution:** 15 minutes before use, centrifuge the concentrated biotinylated antibody at 1000×g for 1 minute, and dilute the 100× concentrated biotinylated antibody into a 1× working concentration (10 µL concentrated + 990 µL universal diluent) in a universal diluent, and use it on the same day.

4. **Preparation of enzyme conjugate working solution:** 15 minutes before use, centrifuge 100× concentrated streptavidin-HRP at 1000×g for 1 minute, and dilute 100× concentrated streptavidin-HRP to 1× working concentration in a universal diluent (10 µL concentrated + 990 µL universal diluent) for use on the same day.

5. **Preparation of 1× washing solution:** take 10ml of 20× washing solution to 190ml of distilled water (the concentrated washing solution taken out of the refrigerator may have crystallization, which is a normal phenomenon, it can be placed at room temperature, shake evenly, and then configure after the crystallization is completely dissolved).

Assay Procedure

1. Remove the desired slats from the foil pouch after 10 minutes of room temperature equilibration and place the remaining slats back at 4°C with a ziplock bag.

2. Loading: Add 100 µl of samples or standards of different concentrations to the corresponding wells and add 100 µL of universal diluent to the blank wells. Incubate for 1 h at 37°C after covering with the plate sealer. (Suggestion: Samples to be tested are diluted by a minimum of 1-fold with a universal diluent before being added to the microplate for testing to reduce the impact of matrix effects on the test results, and the sample concentration should be multiplied by the corresponding dilution factor when calculating the sample concentration. All samples and standards to be tested are recommended to be double-well).

3. Add biotin-antibody: Remove the plate and discard the liquid without washing. 100µL of biotin-antibody working solution was directly added to each well, and incubated at 37°C for 1 hour after covering with a plate sealer.

4. Wash the plate: Discard the liquid, add 300µL of 1x wash solution to each well, let it stand for 1 minute, shake off the wash solution, pat dry on absorbent paper, and repeat the wash 3 times (you can also use a plate washer to wash the plate).

5. Add streptavidin-HRP working solution: Add 100µL of streptavidin-HRP working solution to each well, cover the plate and incubate at 37°C for 30 minutes.

6. Wash the plate: Discard the liquid and wash the plate 5 times according to the washing method in step 4.

7. Add TMB Substrate: 90 µL of TMB Substrate was added to each well, covered with a plate sealer, and incubated at 37°C for 15 minutes in the dark.

8. Add stop solution: Remove the microplate, add 50 µL of stop solution directly to each well, and immediately measure the OD value of each well at a wavelength of 450 nm.

Calculation of Results

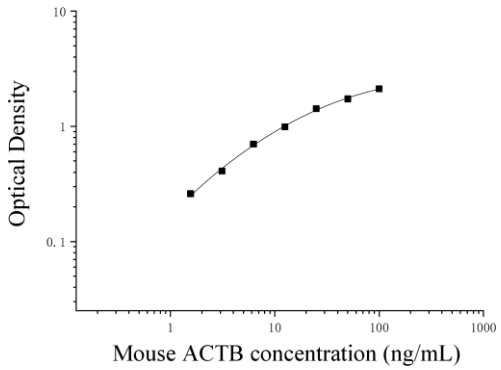
1. Calculate the average OD value of the standard and sample replicate wells and subtract the OD value of the blank well as the correction value. Using the concentration as the abscissa and the OD value as the ordinate, the standard curve of the four-parameter logistic function is drawn on a double-logarithmic coordinate paper (the value of the blank group is removed when plotting).

2. If the OD value of the sample is higher than the upper limit of the standard curve, it should be diluted appropriately and remeasured and multiplied by the corresponding dilution factor when calculating the sample concentration.

The following data and standard curves are for reference only, and experimenters need to establish standard curves according to their own experiments.

Typical Data & Standard Curve

Conc. (ng/mL)	100	50	25	12.5	6.25	3.12	1.56	0
OD value	1.94	1.32	0.78	0.51	0.30	0.18	0.12	0.08
Adjust OD value	1.86	1.24	0.70	0.43	0.22	0.10	0.04	-



Note: This figure is for reference only, and the specimen content should be calculated using the standard curve drawn for the same test standard.

Kit Performance

1. Repeatability: The coefficient of variation within the plate is less than 10%, and the coefficient of variation between plates is less than 10%.

2. Recovery: Recovery was calculated by adding 3 different concentration levels of mouse ACTB to selected healthy mouse serum, plasma, and tissue homogenate.

Sample type	Range	Average recovery
Cell culture supernatant (n=8)	90-105	100

3. Linear dilution: Linearity was assessed by adding high concentrations of mouse ACTB to 4 selected healthy mouse serum, plasma, and tissue homogenate, respectively, and diluting within the standard curve kinetics.

Dilution range	Recovery（%）	Cell culture supernatant
1：2	Range（%）	88-111
	Average recovery（%）	97
1：4	Range（%）	103-118
	Average recovery（%）	108

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. Incubation is carried out in strict accordance with the specified time and temperature to ensure accurate results. All reagents must reach room temperature 20-25°C before use. Refrigerate reagents immediately after use.

2. Incorrect plate washing may result in inaccurate results. Make sure to suction out as much liquid as possible from the wells before adding the substrate. Do not allow the micropores to dry out during the incubation process.

3. Eliminate residual liquid and finger marks from the bottom of the plate, otherwise the OD value will be affected.

4. The substrate chromogenic solution should be colorless or very light in color, and the substrate solution that has turned blue cannot be used.

5. Avoid cross-contamination of reagents and specimens to avoid false results.

6. Avoid direct exposure to strong light during storage and incubation.

7. No reaction reagents should come into contact with bleaching solvents or the strong fumes emitted by such solvents. Any bleaching component will destroy the biological activity of the reaction reagents in the kit.

8. Expired products cannot be used, and components with different article numbers and batch numbers should not be mixed.

9. Recombinant proteins from sources other than the kit may not match the antibody of this kit and may not be recognized.

10. If there is a possibility of disease transmission, all samples should be managed, and samples and testing devices should be handled in accordance with the prescribed procedures.

Safety Notes

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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