SpCas9 ELISA Kit - Instruction

Catalog Number : ARK6622

【Intended use】

The SpCas9 protein residue detection kit independently developed by AREX Biosciences can accurately, specifically and conveniently detect Streptococcus pyogenes Cas9 protein residue in cell culture supernatant.

【Principle】

This kit using double antibody sandwich enzyme-linked immunosorbent detection technology to determinate residues of Cas9 protein in the samples. The capture antibody is coated with 96-well labeling plate and the solid phase antibody is prepared. Then first add the standard and testing samples, and second put a marker on horseradish peroxidase labeled antibody, forming a solid phase antibody-Cas9 protein-labeled antibody sandwich, reaction after washing, then add the chromogenic reaction of the substrate. The substrate under the catalysis of HRP become blue, and under the action of stop solution into the final yellow. Determine its absorbance value (OD value) at wavelengths of 450 nm and 630 nm, where, 630 nm is the corrected wavelength. The OD value is positively correlated to content of Cas9 protein in the sample .

【Materials provided】

Box	Number	Component	Size	Storage condition
Box 1	1	Microtiter Plate	8×12	2-8℃
	2	HRP-Conjugate Antibody(20×)	750 μL×1 tube	2-8℃(keep in dark)
	3	Sample Dilution	60 mL×1 vial	2-8℃
	4	HRP-Conjugate Antibody Dilution	15 mL×1 vial	2-8℃
	5	Wash Buffer (20×)	30 mL×1 vial	2-8℃

	6	Developing solution A	8 mL×1 vial	2-8℃ (keep in dark)
	7	Developing solution B	8 mL×1 vial	2-8℃ (keep in dark)
	8	Stop Solution	15 mL×1 vial	2-8℃
	9	Microplate Sealers	3	-
Box 2	10	Standard (2μg/mL)	100 μL×3 tubes	-20℃

【Storage】

The standard is stored at -20℃, while the other components are stored at 2-8℃. The expiration date is 12 months from the date of manufacture, and the expiration date after opening is 2 months. After opening, the unused kit should be stored at the specified storage conditions.

The date of manufacture and expiration date are shown on the kit label.

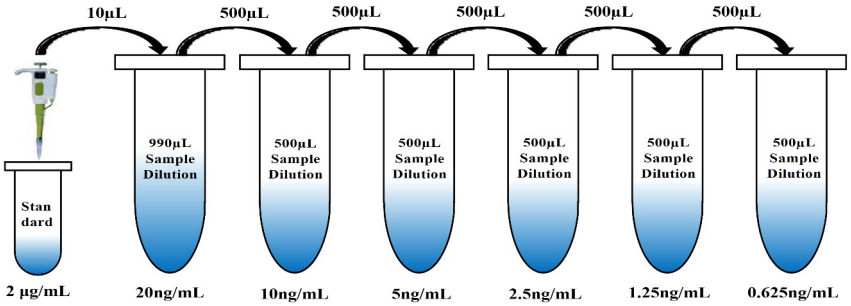
【Reagents/equipments required but not supplied】

1. Enzyme-labeled instrument, thermostatic oscillator (or thermostatic incubator), plate washer (or hand washing), vortex oscillator, timer
2. Pipettes and pipette tips (0.5- 10μL, 10- 100μL, 30-300μL, 100- 1000μL)
3. Deionized or distilled water.
4. Bibulous paper, EP tubes, disposable gloves.

【Reagent preparation】

- Wash Buffer(1×):Take 1 part of Wash Buffer (20×) and add 19 parts of deionized water to prepare a working concentration wash buffer (1×). If there is crystallization in the wash buffer (20×), it should be gently shaken at room temperature or 37℃ in a water bath, and then diluted after the crystallization is completely dissolved. Unused wash buffer (20×) should be stored at 2-8℃.
- HRP-conjugate antibody(1×): add the HRP dilution into the HRP-conjugate antibody(20×)to dilute for 20 fold. For example, add 190 μL of HRP dilution into 10 μL HRP-conjugate antibody(20×), and mix well.
- Substrate Solution: Mix the developing solution A and developing solution B with equal volume before 10 minutes using. Avoid light to ensure that the substrate solution is not contaminated. If the substrate solution after mixing has turned blue, do not use it.

- Standard: The standard (2μg/ml) is diluted to 20ng/ml with the sample diluent, and then the subsequent standards are prepared by serial ratio (dilution: 2-fold) as shown below, duplicate well detection.



- Bring the sample back to room temperature before adding it. Mix well before adding (the sample should be a uniform solution, and if there are cell fragments, they need to be centrifuged and then added for testing). If users need to dilute the sample or high-concentration standards in the kit, they can use the sample dilution solution for dilution.

  Cell supernatant: Centrifuge the sample at 2-8℃ at 1000-2000×g for 10-20 minutes to obtain the supernatant. Test the sample. After collecting the sample, if it is not tested in a timely manner, it is recommended to divide it into smaller quantities for one-time use. After dividing, store it at -70℃ or lower to avoid repeated freezing and thawing.

【Assay procedure】

1. The standard in the kit was placed on the ice box to melt, and the remaining components were placed at room temperature for 30min before operation. remove the required slats from the aluminum foil bag that has been balanced to room temperature, mark the order of the slats with a marker, and put the remaining slats back into the aluminum foil bag with a sealing plate film, seal it, and store it at 2-8℃.

   (Note: In the process of washing the board is easy to fall off, pay attention to mark.

2. Temperature breeding of samples: set up standard wells, blank wells and sample wells. 100μL of standard substances of different concentrations were added to the standard wells in order, and 100μL of Dilution to the blank wells, and 100μL of samples to be tested were added to the sample wells, and then incubate at 37℃ and 500rpm for 1h.

3. Wash the plate: After warming, carefully remove the sealing plate film, discard the liquid in the hole, wash the plate with wash buffer (1×) for 3 times (250μL/hole), and pat the residual liquid in the hole.

(Note: If the washing method is hand washing, it can be left for 1min after adding the wash buffer (1×); If the washing machine is used to wash the board, it can be slightly shaken for 5s after adding the wash buffer (1×).

4. Incubation of HRP-conjugate antibody (1×) : Add HRP-conjugate antibody (1×), 100μL/ well, seal the plate with sealing plate film, and then incubate at 37℃ and 500rpm for 1h.

5. Wash the plate: After warming, carefully remove the sealing plate film, discard the liquid in the hole, wash the plate with wash buffer (1×) for 3 times (250μL/hole), and pat the residual liquid in the hole.

6. Color development: the pre-prepared substrate solution was added to the enzyme label plate, mixed well (100 μL/well), and incubated in darkness ,37℃ for 15 min.

7. Termination: add stop solution (100 μL /well).

8. Recording: The OD value at 450 nm/630 nm should be measured by placing the plate in the microcoder, and the reading should be completed immediately.

【Calculation of results】

1. Calculation of absorbance value: The calibrated value of light absorbance of each standard or sample is: ($OD_{450\ nm}$-$OD_{630\ nm}$)- absorbance value of the blank control well ($OD_{450\ nm}$-$OD_{630\ nm}$) .

2. Plot the standard curve with concentration of the standard as the horizontal coordinate (X) and the calibrated value of light absorption of the standard as the vertical coordinate (Y), and a four-parameter Logistic mathematical model is recommended to fit the equation:Y= （（A-D）/（1+（X/C）^B））+D.Substitute OD value of the sample ($OD_{450\ nm}$ - $OD_{630\ nm}$) - absorbance value of the blank control well($OD_{450\ nm}$-$OD_{630\ nm}$)) into the formula to calculate the content of SpCas9 protein in the sample, and note the dilution factor.

3. If the OD value of the sample to be tested exceeds the highest OD value of the standard curve, the sample shall be redetermined after dilution.

【Precautions】

1. The standard in the kit should be stored at -20℃ after use, and the frequency of freezing and thawing of the standard product should be < 6 times.

2. This kit is only used for in study, not for clinical diagnosis.
3. This kit is only used for research in study and is not for use in clinical diagnosis.
4. This kit should not be used beyond the expiration date.
5. All reagents in the kit must be restored to room temperature (20-25℃) before use.
6. The best detection effect can be guaranteed only by strictly following the operation method of the instructions and using all the reagents matched with the kit.
7. Each test should prepare the corresponding standard curve. DO NOT USE the standard curves from different kits or different batches.
8. Pay attention to the timely replacement of sample adding tank and pipette tips between different samples and steps to avoid cross contamination.
9. The final test results are closely related to the effectiveness of the reagent the operator's method of operation and the test environment.
10. The company is only responsible for the kit itself and is not responsible for the sample consumption caused by the use of the kit. Users are requested to take full account of the possible usage of the sample and reserve sufficient samples before using the kit.

【Safety instructions】

1. All the biological samples have potential biosafety risks, the users shall operate, dispose of and discard the samples in strict accordance with the local laws and relevant provisions.

2. For safety reasons, the operators shall wear the personal protective equipment, such as lab coat, gloves, face masks and safety glasses.

【Problem analysis】

If there is any problem with the experimental results, please take photos of the color rendering results in time, and properly preserve the unused slats and reagents. Then contact technical support to find out the reason.

Problem Description	Possible Reasons	Solution
Gradient difference of	Dilution error	The standard curve is not diluted according to the dilution factor

standard curve	The liquids are aspirated or added inaccurately	Check the pipette and tips
	The ELISA plates are not washed completely	Guarantee the plate washing times, and the amount used for washing liquid per well
The color development is weak or colorless	The incubation time is too short	Guarantee sufficient incubation time
	The experiment temperature is not correct	Use the recommended incubation temperature
	The reagent volume is insufficient or miss to add the reagent	Check the liquid aspiration and adding process, and guarantee all the reagents are added sufficiently in sequence
	The developing solution are prepared wrongly	The developing solutions A and B are mixed in same volume and protected from light, which are prepared 10 min before color development
The OD value are of low reading	The sample is not treated	The samples are tested after centrifugation by heating
	The plate reader are set incorrectly	Check the wavelength and optical filter device on the plate reader
		The plate reader shall be opened in advance before reading for preheating
The OD value are of high reading	The sample is not diluted	It is recommended to validate the dilution factor of the sample
The coefficient of variation (CV value) is large	The liquid adding is incorrect	Check the liquid adding condition
	There are pollution at the bottom of the ELISA plate	Check the bottom of the ELISA plate for residual liquid or handprint
	There are foreign matters or bubbles in the plate well	Confirm that there are no foreign matters in the plate well before adding samples, and confirm that there are no bubbles after adding samples

	The plate is not sealed or sealed incompletely during incubation	Seal the plate with a plate sealer
The background value is high	The ELISA plates are not washed completely	Wash the plate according to the method recommended in the instruction for use
		If automatic plate washer is used, please check all the liquid adding port and waste liquid outlet for blocking
		If the plate is washed by hands, the plate washing times can be added properly
		Insufficient washing or missing to wash may lead to high background
	Incubation time and temperature	Operate in strict accordance with requirements in the instruction for use
	Consumption pollution	The consumables used (such as tubes, tips etc.) are not clean
	The washing solution is polluted	Prepare fresh washing solution
	The developing solution is polluted	The color developing solution itself does not have colors, please ensure that the color developing solution is not polluted by metal ion or oxidizing agent before use, and it is protected from light.
The sensitivity is low	The kit is stored improperly	Store the related reagents in accordance with the requirements in the instruction for use